# ENTEROBACTERIACEAE QUALITY AND DIVERSITY OF VEGETABLES SOLD IN THE JOHANNESBURG METROPOLIS

**by**

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DECLARATION

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Title: Enterobacteriaceae quality and diversity of vegetables sold in the Johannesburg Metropolis.

I declare that the above thesis is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references. I further declare that I have not previously submitted this work, or part of it, for any degree or examination in any other higher education institution.

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## DEDICATION

I dedicate this thesis to God Almighty for giving me the strength and courage to complete this research.

**ABSTRACT**

The contamination of street vended vegetables may occur through the usage of manure and contaminated irrigation water, and the consumption of these vegetables, such as ready-to-eat salads, can cause foodborne diseases in consumers. The objective of this study was to investigate the Enterobacteriaceae diversity in vegetables sold at informal markets in the Johannesburg Metropolis. A total of 201 vegetable samples were purchased from randomly selected street vendors from different regions in the Johannesburg Metropolis and analysed for aerobic growth count and Enterobacteriaceae contamination using Plate Count Agar (PCA), and violet red bile glucose agar (VRBGA), respectively. The diversity of bacterial isolates was analysed using sequencing and phylogenetic analysis. The aerobic bacterial growth counts of vegetables from all the regions ranged from 7.66(±0.759) to 8.37(±0.347) log10 cfu/g and the mean aerobic growth counts of vegetables from Soweto and Yeoville were significantly different ($p \leq 0.05$) from those of the other regions, but were not significantly ($p > 0.05$) different across different vegetable types. The Enterobacteriaceae growth counts in vegetables from all the regions ranged from 5.05 (±0.647) to 5.45 (±0.693) log10 cfu/g. The mean Enterobacteriaceae growth counts of vegetables were not significantly ($p > 0.05$) across each region and different vegetables types. The predominant Enterobacteria genera were *Serratia* (35%), followed by *Hafnia* (21%), *Aeromonas* (17%), and *Pseudomonas* (5%). In conclusion, this study shows that the vegetables sold at the informal markets in the Johannesburg Metropolis have high aerobic bacterial growth and Enterobacteriaceae contamination due to poor hygiene practices. The dominant Enterobacteriaceae genera isolated are *Aeromonas*, *Hafnia*, *Serratia*, and *Pseudomonas*, which

could be opportunistic pathogens. It is recommended that the Department of Health improves vending and sanitation facilities, to prevent cross contamination.

**Key words:** contamination, vegetables, bacteria, growth counts, regions, predominant, hygiene, diversity, informal markets, sequencing.

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| | |
|---|---|
| **DNA** | Deoxyribonucleic Acid |
| **r RNA** | Ribosomal Ribonucleic Acid |
| **PCR** | Polymerase Chain Reaction |
| **CFU** | Colony Forming Unit |
| **PCA** | Plate Count Agar |
| **VRBGA** | Violet Red Bile Glucose Agar |
| **NCBI** | National Centre of Biotechnology Information |
| **CBD** | Central Business District |

# CHAPTER 1:

# INTRODUCTION

## 1.1 BACKGROUND

There are various global awareness programmes, driven by the World Health Organisation (WHO), to promote the consumption of vegetables at specific recommended portions for better health and well-being of humans especially for children and elderly individuals (Rekhy & McConchie, 2014). Green-leafy vegetables such as Chinese cabbage, pigweed, Jews mallow, cowpeas, pumpkin leaves, Tsamma melon, spider flower, and black nightshade are good sources of micronutrients such as potassium, iron, beta carotene (β–carotene), calcium, magnesium, and vitamins such as vitamin A and C (Van Jaarsveld et al., 2014). The consumption of vegetables is increasing on an annual basis globally, considering that there is scientific evidence that the consumption of vegetables may help prevent many degenerative diseases such as cardiovascular and cancer related diseases (Rico et al., 2007). Vegetables contain high amounts of phytochemical compounds such as vitamin A and C, and β–carotene which can prevent tissue and nucleic acid damage due to oxidative stress (Kongkachuichai et al., 2015). These phytochemical compounds regulate the oxidative signalling pathways and protect cells from molecular damage (Chikara et al., 2018).

Apart from their chemical composition and nutritive value, the quality of fruits and vegetables are often iimpacted by a number of spoilage and pathogenic microorganisms (Snyder and Worobo, 2018). The reduction of Enterobacteriaceae and *Pseudomonas* counts on vegetables such as baby spinach can be beneficial in reducing the public health complications caused by

these spoilage organisms on consumers (Truchado et al, 2019). Improper crop growing conditions in the field and improper hygienicpractices when handling vegetables during harvesting, transportation, and storage may enhance contamination and spoilage of vegetables due to growth of microorganisms (Shobha, 2014). Vegetables grown in soil containing treated or raw manure have been found to have a higher risk of being contaminated with antibiotic resistant bacteria (Tien et al., 2017). The contamination of vegetables by bacteria across the farm-to-fork chain has been found to be especially high during transportation (Ssemanda, 2017). During processing, vegetable contamination occurs in the production plants if the surfaces are not properly sanitised and hygiene not properly maintained (Lehto et al., 2011).

Vegetables such as lettuce, broccoli, cucumber, tomato, cabbage, carrot, green pepper, cauliflower, mushroom, green pea, spinach, and onion have been found to have a high prevalence of pathogens such as *E. coli* and *Salmonella* (Sospedra et al., 2013). Both organic and conventional vegetables have been found to contain high levels of pathogens such as *E. coli* due to poor agronomic and hygiene practices during harvest, transportation, and selling of vegetable products (Maffei et al., 2013). Furthermore, vegetables such as lettuce and cabbage, which are often consumed raw, have been found to contain high counts of both pathogenic and spoilage bacteria before and after harvest (Dugassa et al., 2014).

The spoilage of vegetables such as tomatoes is due to contamination with a wide variety of bacteria and fungi, including: *Bacillus subtilis*, *Bacillus aureus*, *E. coli* 0157:H7, *Klebsiella aerogene, Pseudomonas aeroginosa*, *Salmonella typhi*, *Proteus mirabillis*, S*taphylococcus aureus*, *Penicillium* spp, *Mucor* spp., *Aspergillus niger*, *Furasium* spp., and *Saccharomyces cerevisae* (Wogu et al., 2014).

The presence of Enterobacteriaceae in lettuce and curly endive vegetables was shown in a study which investigated the characteristics of Carbapenem-resistance Enterobacteriaceae in ready-to-eat vegetables in China. The Enterobacteriaceae species isolated from these vegetables were *Citrobacter freundii* and *Klebsiella pneumoniae*, which are pathogenic in humans (Liu et al., 2018).

Extended spectrum beta-lactamase (ESBL) -producing *Rahnella aquatilis* and *Serratia fanticola,* the ones that produce penicillin, cephalosporin and cephamycin resistant enzymes (AmpC beta-lactamase -producing) *Hafnia alvei*, *Serratia plymuthica*, and *Citrobacter freundii*, as well as third-generation cephalosporin (3GC) - resistant Enterobacteriaceae were isolated from retail vegetables sold in Dutch stores. Contaminated vegetables may transmit the resistant Enterobacteriaceae to consumers, resulting in them acquiring these resistant genes (Hoek et al., 2015).

## 1.2 PROBLEM STATEMENT

Problems related to microbial contamination of retail vegetables are most often associated with the presence of pathogenic and spoilage organisms. The contamination of vegetables may occur through the usage of manure and contaminated irrigation water on vegetables such as lettuce and spinach (Adenusi et al., 2015). The consumption of vegetables such as ready-to-eat salads, which are contaminated by pathogens, can cause foodborne diseases in consumers (Stephan et al., 2015). *Escherichia coli* O157:H7 has been isolated from lettuce grown on soil treated with contaminated compost and water that was used for irrigation (Faour-Klingbeil et al., 2015). The contamination of vegetables can also occur as a result of poor hygiene practices during

processing, especially washing, peeling, cutting, and packaging (Castro-Ibanez et al., 2017). Kitchen knives used to cut lettuce have also been found to transmit *E. coli* and *L. monocytogenes* from contaminated lettuce to fresh lettuce. (Zilelidou et al., 2014).

Spoilage of vegetables is a great cause of concern to consumers and vegetables juice manufacturers alike (Snyder et al, 2018). *Pseudomonas* species are the most common spoilage bacteria isolated from vegetables such as spinach, lettuce, and broccoli. In packaged vegetables, the most common spoilage bacteria are the gram-positive lactic acid bacteria. Spoilage bacteria can be transmitted to consumers through the ingestion of contaminated fruit and vegetables (Douesset, Jaffres, and Zagorec, 2016). The low cost Neutral Electrolysed Water washing method can reduce contaminating spoilage *Pseudomonas* species from vegetable surfaces and control the effect of their spoilage (Pinto et al., 2015).

## 1.3 IMPORTANCE OF THE STUDY

Despite the various global awareness programmes, driven by the WHO, that encourage the consumption of microbiologically safe vegetables due to their health improving potentials (Rekhy & McConchie, 2014), some studies have revealed that vegetable may be a source of both pathogenic and nosocomial bacteria for humans. The findings from this study will give an overview of the hygiene condition of vegetables sold at informal markets in the Johannesburg metropolis. Data from this research will be provided to the Johannesburg Metropolis Department of Health and this will assist in the development of policies and regulations aimed at improving the sanitary conditions in vegetable retail outlets and improve the hygiene quality of the retail vegetables.

## 1.4 AIM, OBJECTIVES, AND HYPOTHESIS

The aim of this research is to investigate the Enterobacteriaceae quality and diversity in vegetables sold at informal retail outlets in the Johannesburg Metropolis.

The objectives of this research are:

1. To investigate the microbial quality of prominent vegetables sold in different locations within the Johannesburg Metropolis.

2. To investigate the Enterobacteriaceae quality and diversity in vegetables sold at informal retail outlets in the Johannesburg Metropolis.

3. To investigate the phylogenetic relationship of Enterobacteriaceae species isolated from vegetables sold at informal retail outlets in the Johannesburg Metropolis.

The research hypothesis are:

1. The aerobic bacterial growth of different types of vegetables sold at informal retail markets will be similar or higher than log10 cfu/g.

2. The enterobacteria growth count of different types of vegetables sold in informal retail outlets in the Johannesburg Metropolis will be similar or higher than 3log cfu/g.

3. The enterobacteria species in different vegetable types sold at the informal retail outlets in the Johannesburg Metropolis will be diverse.

## 1.5 DISSERTATION LAYOUT

This study is made up of six chapters, organised as follows:

Chapter 1: Introduction

This is an introductory chapter to the study, it provides background and an overview of the research. Included in this chapter is the problem statement, purpose of the study, aim, and objectives.

Chapter 2: Literature review

This chapter gives an overview of existing literature on the microbiology of vegetables.

Chapter 3: Research methodology

This chapter provides details of the locality of the study, method of sampling, data collection method, and instruments used.

Chapter 4: Results

This chapter provides the collected results and its detailed description.

Chapter 5: Discussion

This chapter provides the explanation and interpretation of the results, based on previous studies.

Chapter 6: Conclusion and recommendations

This chapter provides the summary of the research findings based on the objectives and possible solution to the research problem.

# CHAPTER 2:

# LITERATURE REVIEW

## 2.1 COMMERCIALISATION OF FRUITS AND VEGETABLES

Vegetables such as lettuce, cucumber, and tomato are used to make raw vegetable salads sold at both formal and informal markets. These vegetables are washed and shredded to produce salads (Sopedra et al., 2013). Some fruits and vegetables are canned before being sold to consumers (Durand et al., 2015). International standards for fruits and vegetables have been introduced as these products become commercialised. For example, methods of mycotoxin screening are being used for identification of mold on tomatoes, onions and other fruits (Van der Perre et al., 2014). In Nigeria, indigenous fruits such as African star apple, African mango, hog plum, Roselle, and tamarind have been commercialised on a small scale. These fruits are used to produce juices, jams, and other drinks (Aworth, 2015). Global harmonisation of food safety regulations has improved compliance with food safety requirements over the years (Hou et al., 2015).

## 2.2 THE CONSUMPTION OF FRUITS AND VEGETABLES

Fruit and vegetable consumption has been on the increase worldwide, both in children and adults. When portions of vegetables are increased in the children's lunch packs, quantities consumed also increase (Miller et al., 2015). Eco-labelling has also resulted in consumers preferring such products as they are more informative (Sillani et al., 2015). In South African markets, healthy foods are costly, and this reduces their consumption. A study carried out in the Western Cape indicated that in rural areas the markets that sell these foods are mostly unknown

to most people who are relatively poor when compared to urban dwellers (Temple et al., 2011). Wild South African vegetables are a good source of nutrients. However, these are only commonly available and consumed in approximately five out of the nine provinces. Some individuals still feel that these vegetables are only for the poor or low-class individuals and this reduces their popularity and consumption (Bvenura et al., 2015)

## 2.3 PROCESSED FRUIT AND VEGETABLE PRODUCTS

Raw fruit and juices are prepared by crushing or squeezing fresh fruits, and in some cases without being pasteurised during processing (Simforian et al., 2015). Vegetable salads are a vehicle of antibiotic resistant pathogens which pose a risk of foodborne illnesses to consumers (Campos et al., 2013). In both formal and informal markets in South Africa, common fruit and vegetable products include beverages such as fruit juices and rooibos tea respectively (Van Wvk, 2011), fruit salads, cooked broccoli, carrots, green leafy vegetables such as spinach, and more traditional vegetables such as cowpeas, pumpkin leaves, and spider plant (Kruger et al., 2015).

During processing of Portuguese ready-to-eat salads, lack of proper hygiene and disinfection resulted in transmission of pathogenic bacteria such as *Salmonella, Listeria monocytogenes* as well as antibiotic-resistant Enterobacteriaceae such as *Raoultella terrigena* and *Citrobacter freundii* (Campos et al, 2013). The contamination of salads may result in the transmission of bacteria and their characteristics to consumers (Campos et al., 2013). *Listeria monocytogenes* was also isolated from patients from different states in Switzerland who had consumed ready-to-eat salads produced by a salad-producing company in the country. The presence of this microbial

contaminant was as a result of improper hygiene practices at one of their production lines (Stephan et al., 2015).

## 2.4 MICROBIOLOGICAL SPOILAGE OF PROCESSED FRUIT AND VEGETABLE PRODUCTS

Dried onion products and garlic powder contain lactic acid bacteria, which cause spoilage of these vegetables. The *Weissella* bacteria species have been found to be the most prevalent pathogens in these products (Sade et al., 2015). Aneja et al. (2014) found *Aspergillus flavus*, *Candida* spp., *Cladosporium* spp., *Acetobacter* spp., and *E. coli* in freshly prepared citrus and carrot juices.

In a study designed to determine the incidence and impact of microbial contamination on the spoilage of fruit and vegetable juices in USA, the manufacturers reported several cases of microbial spoilage of juices resulting from the presence of *Alicyclobacillus* spp. and mold. They indicated that there is an urgent need for the implementation of proper sanitation and production quality control processes to reduce such cases (Snyder and Worobo, 2018).

Bacterial species such as *Salmonella* and *E. coli* are the most common pathogens transmitted through fruit and vegetable products (Amrutha et al, 2017). Heat treatment during processing can eliminate these bacteria but some *Clostridium* spp. and *Bacillus* spp. are resistant to heat, indicating that other processing techniques may be required to eliminate these pathogens. When high hydrostatic pressure is used during processing, its effectiveness to eliminate *E. coli* and *Salmonella* is reduced by the matrix in the fruits and vegetables (Nguyen-the, 2012).

## 2.5 CONTAMINATION OF FRUITS AND VEGETABLES

### 2.5.1 Pre-harvest contamination

During production, fruits and vegetables may be contaminated through use of untreated water for irrigation, as well as the use of organic fertilisers such as manure. Waste municipality water contains a high microbial count which, if not properly treated, may transmit the pathogens to the plants and the soil (Vivaldi et al., 2013).

A study to compare the prevalence of pathogens in plants watered using differently treated waste water used for irrigation of nectarine plants over a period of two years, showed that water from secondary treatment processes had a higher microbial count, resulting in a high prevalence of pathogens in nectarine fruits (Vivaldi et al., 2013). Water treatment processes may not eliminate all the pathogens therefore, water quality is of utmost importance in the prevention of contamination of fruits and vegetables. Irrigation water is also a vehicle for transmission of *E. coli* O157:H7 during the production of vegetables such as lettuce. Even if irrigation is stopped three days prior to harvest, it does not eliminate the pathogens (Alam et al., 2015). *E. coli* from the irrigation water has also been detected in the soil in which the water is used (Oliveria et al., 2012) and have been found to survive in the leaves of baby lettuce plants at the time of harvest and commercialisation (Chitarra et al., 2014). In developing countries, wastewater is used to ensure the availability of vegetables. However, there is a higher risk of contamination and chances of foodborne disease outbreaks (Barker et al., 2012).

The contamination of vegetables may occur through the use of organic fertilisers. Some organic fertilisers, such as animal manure, may contain fecal pathogens such as *Listeria monocytogenes*,

*E. coli*, *Salmonella* spp., and *Campylobacter*. These pathogens are then transmitted to plants during production. A study conducted on the microbial analysis of organic and conventional vegetables such as lettuce, chicory, collard greens, and arugula, during which samples were analysed for mesophilic anaerobic bacteria, yeast and molds, total coliforms, *E. coli*, and *Salmonella* spp. showed that the organically grown vegetables had higher microbial counts than the conventional vegetables (Maffei et al., 2013). The use of contaminated compost during the production phase increases the prevalence of pathogens in plants such as, especially lettuce (Oliveria et al., 2012).

### 2.5.2 Post-harvest contamination

Post-harvest contamination occurs during handling, storage, and processing of fruits, vegetables, and related products. During, for example, salad production the fresh fruits and vegetables are washed beforehand. If contaminated water is used during this process, cross contamination may occur. For example, a study to determine the cross-contamination of *E. coli* O157:H7 between lettuce and wash water, showed that the leafy green vegetables can be contaminated by contaminated water used for washing. When this water was re-used to wash fresh leaves, cross-contamination occurred (Jensen et al., 2015).

During cutting and shredding of leafy green vegetables cross contamination may occur from the usage of contaminated utensils. Usually no sanitisers are used on knives and other shredding equipment during processing. The contaminated knives then transmit pathogens such as *E. coli* and *Listeria monocytogenes* to the fresh leaves (Zilelidou et al., 2015). Salads are minimally processed and therefore, the pathogens can survive and may be transmitted to consumers. Graters

and knives may also become contaminated with *E. coli* and *Salmonella* when used to cut contaminated tomatoes and carrots (Erickson et al., 2015). During canning, the microbial spores accumulate in blanchers and conveyors, thereby causing a risk of contamination of canned fruits and vegetables with heat-resistant spores (Durand et al., 2015).

Food handlers may transmit pathogens to food due to poor hygiene. Proper handwashing, brushing, sanitization, and the use of gloves are always a requirement, and all the utensils and hard surfaces need to be sanitised frequently. Todd (2014) stressed the importance of thoroughly cleaning all parts of the hands that could harbour fecal pathogens, during handwashing practices. In some cases, vendors do not have access to running water and as such never wash their hands after handling money or using the toilet. This results in the transmission of pathogens to the fruits, vegetables, or other prepared food which they sell.

## 2.6 SURVIVAL OF PATHOGENS IN FRUITS AND VEGETABLES

**Post-harvest contamination**: Pathogens inhabit the surfaces of fruits and vegetables, and some pathogens such as *Salmonella enterica* attaches more readily to lettuce than cabbage (Patel et al, 2010). Pathogens which bind on the surfaces of fruits and vegetables can gain entry into the tissue of these plants when the protective epidermal layer is damaged. These pathogens survive on the juices that ooze from the damaged plant tissue (Pilizota 2014; Olaimat et al, 2012). Furthermore, the skin vegetables can be damaged during production or harvest, creating favourable conditions for the infestation of bacteria and their spores (Elhariry, 2011).

**Internalisation**: When fruits and vegetables are washed with contaminated water the pathogens may eventually penetrate the inner tissue and proliferate. For example, in a study carried out to

investigate the internalisation of *Salmonella* in tomatoes, the inoculum was placed at the stem scar and bacteria proceeded to migrate to the inner tissue and proliferate (Bartz et al., 2015). When this happens, bacteria remain in the fruit, even when the tomatoes are washed or sanitised during processing. This may result in infection of the consumers. *Salmonella* internalisation has also been observed in lettuce due to extreme weather stress (Ge et al., 2014).

## 2.7 SURFACE DISINFECTION OF FRUITS AND VEGETABLES

Measures to control or reduce contamination of fresh fruits and vegetables have been applied in various cases. Many bio-control agents have been isolated and tested for use in eliminating pathogens on fruits and vegetables without much alteration of their quality (Siroli et al., 2015). Washing with chlorine water has been found to be more effective and less costly in reducing microbial counts on fruits and vegetables (Ramos et al., 2013). In some cases, ozone-based washing is also applied and has proven to be effective (Sillani et al, 2015). Extracts from grape stems have microbial disinfectant properties and can be used to disinfect raw vegetables (Vazquez-Armenta et al, 2017). The utilisation of food irradiation technology during processing has been shown to improve the shelf-life of vegetables when stored at temperatures higher than 10℃, which could reduce the costs and necessity for cold storage (Banerjee et al, 2016). Bacterial sensitivity to disinfectant treatment varies between different pathogens (Scarlett et al, 2016). Vegetable contamination of cut vegetables during washing can also be reduced by repetitive electrolysis of washing water.

## 2.8 ENTERIC BACTERIA FOUND IN VEGETABLES

Irrigated fresh vegetables are a potential transmitter of pathogens from the production field to the consumers. In a study investigating the presence of pathogenic bacteria in irrigation water and vegetables, the results indicated that the prevalence of pathogens in irrigated vegetables increased because of poor quality irrigation water used (Akinde et al., 2016). From this study pathogens isolated from vegetables and irrigated water respectively, were similar and these included different species of *Citrobacter*, *E. coli*, *Enterobacter*, *Klebsiella*, and *Pseudomonas*. Organisms that belong to the Enterobacteriaceae family were isolated from lettuce in Southwest Nigeria. The isolated enterobacteria antibiotic resistance properties, which could be transferred to other organisms including humans was tested and the results showed all the isolates were resistant to cloxaxillin, erythromycin and other antibiotics (Igbeneghu and Abdu, 2014). In a study to determine the prevalence of ESBL-producing Enterobacteriaceae in raw vegetables in Amsterdam, Netherlands, the results showed that enterobacteria could be the source of antibiotic resistance genes found in humans (Reuland et al, 2014). Enterobacteriaceae isolated from vegetables from 18 cities in China were contaminated with carbapenem-resistant Enterobacteriaceae, which could be a serious food safety issue (Lui et al, 2018). If not purposefully managed, this could become a global food crisis.

## CHAPTER 3:

## RESEARCH METHODOLOGY

### 3.1 BRIEF DESCRIPTION OF THE STUDY AREA

This study focuses on the Johannesburg Metropolis with special attention on Hillbrow, Yeoville, Johannesburg CBD, Soweto, and Roodepoort (Figure 3.1). The research areas were selected since there have been a significant increase in the number of vendors selling different types of fruits and vegetables at the informal markets in these areas. The vendors in Hillbrow, Johannesburg Central Business District (CBD), and Roodepoort areas have their informal vending stalls by the roadside with no toilets or running water nearby, while the Soweto and Yeoville vendors have stalls in open, informal markets. It has been observed that various vegetables are regularly dipped in the same water bucket to keep them moist.


**Figure 3.1: Map of the Johannesburg Metropolis**

(Adapted from https://municipalities.co.za/map/2/city-of-johannesburg-metropolitan-municipality)

## 3.2 RESEARCH DESIGN

A cross-sectional research design was used to randomly collect different types of vegetable samples from different regions within the Johannesburg Metropolis. The reason for choosing this design is that it is affordable, sampling is not spread over a long period of time, and it allows for the study of multiple vegetables from different regions at the same time (Arnett et al, 2017). A quantitative study was conducted in which microbiological analyses were conducted for data collection.

## 3.3 SAMPLE COLLECTION

A total of 201 vegetable samples, consisting of broccoli (9), cabbage (36), cauliflower (9), chomolia (34), giant English rape (29), lettuce (33), Indian kale (14), and spinach (37) were purchased randomly from different vendors at informal markets in Hillbrow, Yeoville, Johannesburg CBD, Soweto, and Roodepoort in the Johannesburg Metropolis. The samples were transported in sterile plastic bags to the laboratory for analyses. Sample collection from the same locations was repeated four times on different days. The collection and analyses of samples carried out from July 2016 to December 2018.

## 3.4 SAMPLE PREPARATION FOR MICROBIAL ANALYSES

Between 50-80g of each vegetable sample was aseptically weighed and mixed with 200ml sterile buffered peptone water (Biolab, South Africa), and then homogenised in a sterilised Waring laboratory blender for 4-8 minutes. Thereafter, ten-fold serial dilutions, up to $10^{-5}$ folds of the homogenate were prepared for each sample and used for bacterial analysis.

### 3.5 AEROBIC COLONY COUNTS

Total aerobic counts (TACs) were done on Plate Count Agar (PCA) plates (Merck, South Africa) using the manufacturer's protocol. Ten-fold serial dilutions of each sample, up to $10^{-5}$ were plated in duplicate and incubated at 37℃ for 24 hours. Plates showing 30-300 colony forming units (cfu) were counted.

### 3.6 ENTEROBACTERIACEA COUNTS

Enterobacteriaceae counts were conducted using violet red bile glucose agar (Merck, South Africa) using the manufacturer's protocol. Ten-fold serial dilutions of each sample up to $10^{-5}$ were plated in duplicates and incubated at 37 ℃ for 24 hours. Round, purple-pink, 1-2 mm diameter colonies surrounded by purple haloes were counted.

### 3.7 EXTRACTION OF BACTERIA DNA

Prior to DNA extraction, a pure culture of each bacteria isolate was prepared by streaking individual colonies of each bacteria isolate PCA agar plate and incubated for 24 hours at 37℃. The carpet of bacteria growth was scraped using a sterile loop and used for DNA extraction. Genomic DNA was extracted from each bacterial culture using the Sigma-Aldrich GenElute Bacterial Genomic DNA Kit (Merck, South Africa). Extraction was done following the maufacturer's protocol. The DNA was quantified using the BioDrop Duo (7444V2.0.2), following the manufacturer's protocol.

### 3.8 AMPLIFICATION OF THE 16S rRNA GENE OF BACTERIA ISOLATES

The amplification of the 16S rRNA gene was done using the universal primers 27F (5'-TCC GTA GGT GAA CCT GCG G-3') and 1492R (5'-TCC TCC GCT TAT TGA TAT GC-3') that cause hybridisations at the end and beginning of 18S and 28S rDNA respectively (Lane et al., 2001). The primers used were synthesised by Inqaba Biotechnology Company (South Africa).

PCR amplification was done in a 25 µl reaction volume, which contained 2 microlitre (µl) template DNA, 0.25 µl forward primer, 0.25µl reverse primer, 0.25µl BSA, 9.75 µl nuclease free water, and 12.5 µl 2x PCR Master Mix (50 units/ml of Taq DNA polymerase in a buffer, pH 8.5), 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP, 3mM $MgCl_2$) (Promega, Madison, USA). Amplification was carried out in a thermocycler (BIO RAD T100) under the following PCR cycler conditions: initial denaturation at 94$^{o}$C for five minutes, six cycles of denaturation at 94$^{o}$C for one minute, annealing at 55$^{o}$C for one minute, extension at 72 $^{o}$C for one minute, the denaturation, annealing and extension steps were repeated for another 32 cycles and the final enlongation at 72 $^{o}$C for 10 minutes.

For gel electrophoresis the wide Mini-sub R cell GT was used (Bio- Rad Laboratories CA, USA). A 1% agarose (Sigma) gel was prepared with two drops of ethidium bromide used for staining. Of the individual PCR products 5-10 µl was used, together with 3 µl loading buffer was pipetted in each agarose gel and well submerged in 0.5 x TAE buffer (Sigma), and the gel was run at 120 V for 35 minutes. GelDoc-It $^{TM}$ 310 Imaging system (California, USA) was used to visualise the DNA strands. Distinctive bands was observed which corresponded with the molecular ladder used.

For samples with very faint bands, the PCR was repeated using the PCR products of the sample as template DNA for the reaction. This produced very visible bands when the gel was visualised under UV light.

### 3.9 IDENTIFICATION OF ENTERIC BACTERIAL ISOLATES BY SEQUENCING

The PCR amplicons products were sent to Inqaba Biotechnology Company (South Africa) for sequencing. The resulting sequences were aligned using Chromas (version 2.6.5) and manually edited to remove the poor quality value bases and correct errors on peak labelling based on colours of the chromatogram. Only peaks with quality values of 30 or more were accepted. The edited sequences obtained and a Basic Local Alignment Search Tool (BLAST) search was conducted to compare the obtained sequences with the nucleotide sequences in the National Center for Biotechnology Information (NCBI) database. Species identification was considered at sequence similarity ≥ 99% ( Lange et al, 2015).

### 3.10 DATA ANALYSIS

Statistical analysis was performed using Microsoft Excel. The aerobic counts were expressed as mean ± standard deviation. The Analysis of Variance (ANOVA) was used to determine the significant difference of the mean aerobic count of vegetables at $p \leq 0.05$. The percentage distribution of Enterobacteriaceae and percentage distribution of four of the most dominant Enterobacteriaceae in all the eight vegetable types, in the five regions of the Johannesburg Metropolis were calculated and expressed in the form of bar graphs and pie charts using Microsoft Excel.

## 3.11 ETHICAL CONSIDERATION

The Johannesburg Metropolis provided permission to conduct this food safety study and ethics clearance was granted by the ethics committee of the College of Agriculture and Environmental Sciences, University of South Africa.

## 3.12 LIMITATION OF THE RESEARCH

The limitation of this study was that there were delays in sample collection and analysis in 2016 due to lack of adequate funding. In 2017 and 2018 when funding from the university was available, the laboratory work could only be conducted in the evenings and weekends and was at times delayed due to work related travelling out of Johannesburg, which lead to the delay in completion of this research.

# CHAPTER 4:

# RESULTS

## 4.1 AEROBIC GROWTH COUNTS OF VEGETABLES IN DIFFERENT REGIONS IN THE JOHANNESBURG METROPOLIS

The results show that the mean aerobic growth count (Figure 4.1) of vegetables sold in the Johannesburg CBD and Hillbrow regions are significantly ($p \leq 0.05$) different from those sold in Yeoville and Soweto. The vegetables from Roodepoort is not significantly different from those in the other regions (Table 4.1). Alternatively, the aerobic colony count of different leafy vegetable types sold the Johannesburg Metropolis were not significantly ($p>0.05$) different (Table 4.2).


**Figure 4. 1: Aerobic growth of a cabbage sample at a dilution of $10^{-5}$ on Plate Count Agar.**

**Table 4.1: Aerobic colony count of different leafy vegetables types sold in different regions of the Johannesburg Metropolis**

| Regions | Minimum | Maximum | Mean Log CFU/g ±Std. Deviation |
|---|---|---|---|
| JHB CBD (n = 51) | 6.13 | 9.17 | 7.70$^{a}$ (± 0.710) |
| Hillbrow (n = 28) | 5.92 | 8.75 | 7.66$^{a}$ (± 0.759) |
| Roodepoort (n = 47) | 7.17 | 8.89 | 7.94$^{ab}$ (± 0.420) |
| Yeoville (n = 50) | 7.04 | 11.15 | 8.23$^{b}$ (± 0.616) |
| Soweto (n = 25) | 7.68 | 9.20 | 8.37$^{b}$ (± 0.347) |
| NB: Mean values with similar letters a or b, are not significantly ($p > 0.05$) different otherwise, they are significantly ($p \leq 0.05$) different. | | | |

**Table 4.2: Aerobic colony count of different leafy vegetable types sold in the Johannesburg Metropolis**

| Vegetable types | Minimum | Maximum | Mean Log CFU/g (±Std.Deviation) |
|---|---|---|---|
| Cabbage (n = 36) | 5.92 | 9.09 | 8.06$^{a}$ (± 0.707) |
| Lettuce (n = 33) | 6.32 | 11.15 | 8.19$^{a}$ (± 0.956) |
| Cauliflower (n = 9) | 7.09 | 9.17 | 8.06$^{a}$ (± 0.621) |
| Broccoli (n = 9) | 7.04 | 8.38 | 7.64$^{a}$ (± 0.525) |
| Spinach (n = 37) | 6.17 | 8.89 | 7.94$^{a}$ (± 0.610) |
| Indian Kale (n = 14) | 6.94 | 8.60 | 7.72$^{a}$ (± 0.526) |
| Chomolia (n = 34) | 6.50 | 8.51 | 7.87$^{a}$ (± 0.463) |
| Giant English Rape (n = 29) | 6.86 | 8.71 | 7.84$^{a}$ (± 0.532) |

NB: Mean values with similar letters a or b, are not significantly ($p > 0.05$) different otherwise, they are significantly ($p \leq 0.05$) different.

## 4.2 ENTEROBACTERIACEA QUALITY OF VEGETABLES IN THE JOHANNESBURG METROPOLIS

The Enterobacteriaceae counts (Figure 4.2) of vegetables form each of the five regions in the Johannesburg Metropolis were not significantly ($p > 0.05$) different (Table 4.3). Similarly, the Enterobacteriaceae count of all eight types of leafy vegetables from the Johannesburg Metropolis were not significantly ($p > 0.05$) different (Table 4.4).

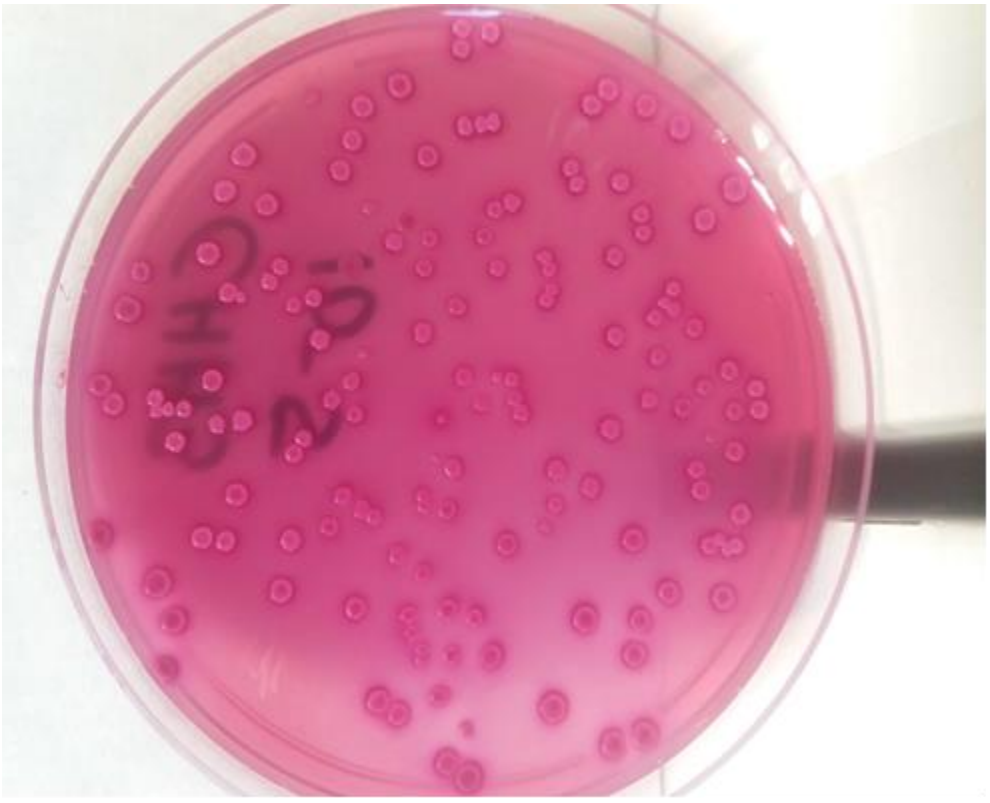

**Figure 4. 2: Enterobacteriaceae growth of a chomolia vegetable sample at a ten-fold serial dilution of $10^{-5}$ on VRBD agar.**

**Table 4.3: Enterobacteriaceae count of vegetables sold in different regions of the Johannesburg Metropolis**

| Region | Minimum | Maximum | Mean Log CFU/g± Std. Deviation |
|---|---|---|---|
| JHB CBD(n = 51) | 3.86 | 7.61 | 5.22[a] (± 0.654) |
| Hillbrow (n = 28) | 4.35 | 6.34 | 5.27[a] (± 0.587) |
| Roodepoort (n = 47) | 3.76 | 6.82 | 5.25[a] (± 0.636) |
| Yeoville (n = 50) | 3.98 | 8.60 | 5.24[a] (± 0.835) |
| Soweto (n = 25) | 4.10 | 5.99 | 5.46[a] (± 0.4410 |
| NB: Mean values with similar letters a or b, are not significantly ($p > 0.05$) different otherwise, they are significantly ($p \leq 0.05$) different. | | | |

**Table 4.4: Enterobacteriaceae count of different vegetable types sold in the Johannesburg Metropolis**

| Vegetable types | Minimum | Maximum | Mean Log EPC count ±Std. Deviation |
|---|---|---|---|
| Cabbage (n = 36) | 4.21 | 7.79 | 5.45[a] (± 0.693) |
| Lettuce (n = 33) | 4.38 | 7.61 | 5.42[a] (± 0.623) |
| Cauliflower (n = 9) | 4.26 | 5.99 | 5.17[a] (± 0.514) |
| Broccoli (n = 9) | 4.22 | 6.04 | 5.08[a] (± 0.571) |
| Spinach (n = 37) | 4.08 | 6.27 | 5.08[a] (± 0.523) |

| | | | |
|---|---|---|---|
| IndianKale (n = 14) | 3.86 | 6.17 | 5.35ª (± 0.615) |
| Chomolia (n = 34) | 4.37 | 6.82 | 5.28ª (± 0.601) |
| GiantEngRape (n = 29) | 3.76 | 6.55 | 5.05ª (± 0.647) |
| NB: Mean values with similar letters a or b, are not significantly ($p > 0.05$) different otherwise, they are significantly ($p \leq 0.05$) different.<br>EPC= Enterobacteriaceae plate count | | | |

## 4.3 IDENTIFICATION OF ENTEROBACTERIACEAE BACTERIAL ISOLATES

Purified DNA of size 780kb (Figure 4.3) were sequenced and BLAST search on the NCBI database was conducted to obtain the identities of the isolates. The sequencing and blasting results indicated a wide range of different enteric bacteria identified from the different types of vegetables (Table 4.5).

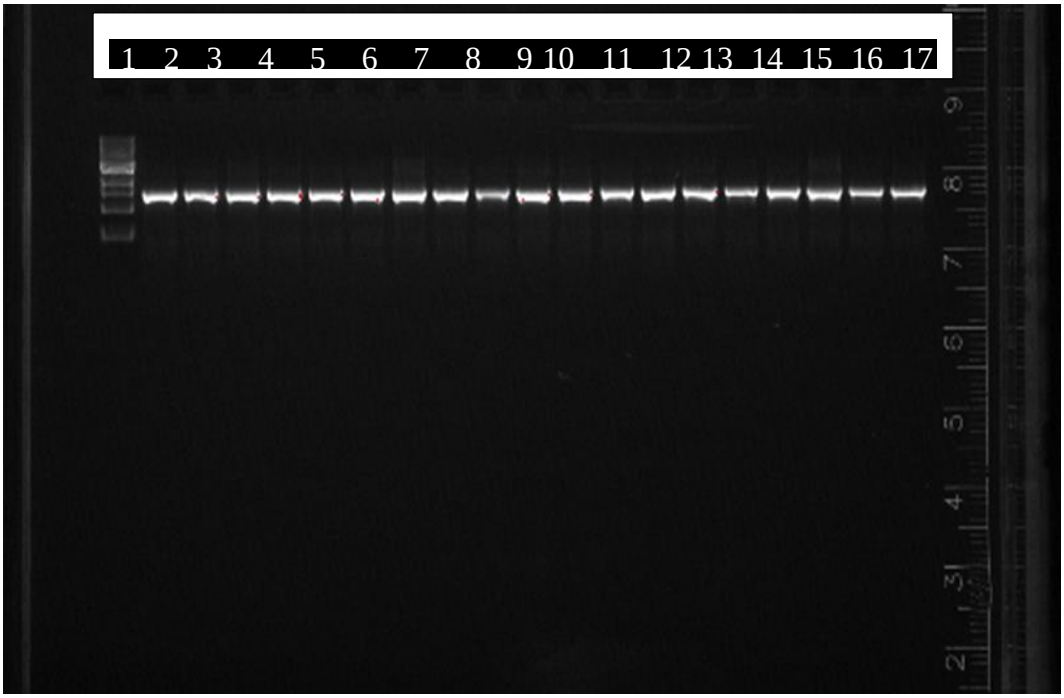

**Figure 4. 3: The electromicrograph the DNA of Enterobacteriaceae isolated from vegetables sold at the informal markets in the Johannesburg Metropolis visualised under UV light using the GelDoc-It TM 310 Imaging system**

Column 1 = 1 kb molecular ladder (Sigma), 2 = *Serratia liquefaciens* strain RHMR 21, 3 = *Serratia liquefaciens* strain FC6859, 4 = *Serratia liquefaciens* ATCC 27592, 5 = *Serratia liquefaciens* strain RHMR 21, 6 = *Serratia liquefaciens* strain LZ-24, 7 = *Serratia liquefaciens* strain AL117, 8 = *Serratia liquefaciens* strain LZ-24, 9 = *Serratia liquefaciens* strain AL117, 10 = *Serratia liquefaciens* strain AK-25, 11 = *Lelliottia amnigena*, 12 = *Serratia liquefaciens* strain FC6859, 13 = *Serratia liquefaciens* strain RHMR 21, 14 = *Serratia liquefaciens* strain RHMR 21, 15 = *Serratia liquefaciens* strain AL117, 16 = *Serratia liquefaciens* strain AL11, 17 = *Morganella morganii* strain CU-BS1

**Table 4.5: The sequencing and blasting results of different vegetable types sold at various markets in Johannesburg Metropolis**

| Sample number | Isolate Identity (Similarity index) | Accession number |
|---|---|---|
| 2b | *Rahnella aquatilis strain PRE12* | MG966290 |
| 3a | *Pseudomonas rhodesiae strain 67B5* | MG269719 |
| 5a | *Enterobacter kobei strain CAU1106* | MF428777 |
| 6a | *Aeromonas salmonicida subsp. pectinolytica 34me* | CP022426 |
| 6b | *Rahnella sp. strain AL187* | MG819528 |
| 7b | *Rahnella variigena strain B7* | MF083084 |
| 8a | *Serratia fonticola strain GS2* | CP013913 |
| 9a | *Aeromonas sp. strain L23* | MH381782 |
| 9b | *Aeromonas sp. AKB-2008-HE79* | AM989244 |
| 12a | *Serratia liquefaciens ATCC 27592* | CP006253 |
| 14a | *Hafnia sp. CBA7124* | AP017469 |
| 15a | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 16b | *Aeromonas hydrophila subsp. hydrophila* | AB680357 |
| 18a | *Shewanella putrefaciens strain PF 15* | KY614355 |
| 18b | *Hafnia alvei strain CBA7135* | CP021971 |

| | | |
|---|---|---|
| 19a | *Hafnia alvei strain CBA7135* | CP021971 |
| 20a | *Rahnella aquatilis strain PRE12* | MG966290 |
| 21a | *Aeromonas sp. strain L23* | MH381782 |
| 22a | *Hafnia alvei strain 14* | KY849243 |
| 26b | *Pseudomonas rhodesiae strain 67B5* | MG269719 |
| 28a | *Hafnia alvei strain FC2951* | MH532496 |
| 29a | *Aeromonas salmonicida subsp. achromogenes strain HLD01* | MH478113 |
| 30a | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 31a | *Serratia liquefaciens strain TPD7002* | MH190215 |
| 31b | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 33a | *Serratia sp. strain 11M5* | KY611630 |
| 34a | *Hafnia paralvei strain FDAARGOS_158* | CP014031 |
| 35a | *Hafnia paralvei strain FDAARGOS_158* | CP014031 |
| 36a | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 36b | *Aeromonas salmonicida subsp. salmonicida* | AM296501 |
| 37b | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 38a | *Hafnia paralvei strain FDAARGOS_158* | CP014031 |
| 38b | *Hafnia paralvei strain FDAARGOS_158* | CP014031 |
| 39a | *Aeromonas salmonicida subsp. salmonicida* | AM296501 |
| 40a | *Aeromonas sobria JCM 2366* | MH381782 |
| 41a | *Rahnella aquatilis strain PRE12* | MG966290 |
| 42a | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 43a | *Hafnia sp. strain 35PMBRU* | KY643489 |
| 44a | *Hafnia paralvei strain FDAARGOS_158* | CP014031 |
| 45a | *Hafnia alvei strain FC2951* | MH532496 |
| 47a | *Aeromonas hydrophila subsp. hydrophila* | AB680357 |
| 48a | *Aeromonas sp. strain L23* | MH381782 |
| 50a | *Serratia liquefaciens strain FC6859* | MH497592 |
| 53a | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 54a | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 55a | *Aeromonas salmonicida subsp. achromogenes strain HLD01* | MH478113 |

| | | |
|---|---|---|
| 56b | *Klebsiella oxytoca strain PF 104* | KY614352 |
| 57a | *Uncultured bacterium clone Shelves_A_100* | MF092425 |
| 61a | *Pseudomonas sp. UYFA249* | KP704434 |
| 62a | *Hafnia alvei strain CBA7135* | CP021971 |
| 63a | *Hafnia alvei strain 14* | KY849243 |
| 65a | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 65b | *Serratia liquefaciens strain AL117* | MG819252 |
| 66a | *Serratia liquefaciens strain AK-25* | KY863496 |
| 67a | *Pseudomonas putida strain S975* | KX817236 |
| 68a | *Pseudomonas sp. J1.2E4* | KF317746 |
| 70a | *Hafnia alvei strain FC2951* | MH532496 |
| 70b | *Serratia liquefaciens strain AL117* | MG819252 |
| 71a | *Hafnia alvei strain FC2951* | MH532496 |
| 71b | *Serratia liquefaciens strain LZ-24* | KU950364 |
| 72a | *Pseudomonas protegens strain TPD3011* | MH221127 |
| 72b | *Serratia sp. HX-B01* | KF501474 |
| 73a | *Pseudomonas protegens strain TPD3011* | MH221127 |
| 73b | *Serratia liquefaciens strain FDAARGOS_125* | CP014017 |
| 74a | *Lelliottia amnigena partial* | CP028520 |
| 74b | *Pseudomonas protegens strain TPD3011* | MH221127 |
| 75a | *Hafnia alvei strain FC2951* | MH532496 |
| 75b | *Serratia plymuthica strain BSW-12* | KX901796 |
| 76a | *Raoultella ornithinolytica strain FDAARGOS_431* | CP023888 |
| 77a | *Serratia liquefaciens strain AL117* | MG819252 |
| 78a | *Hafnia alvei strain FC2951* | MH532496 |
| 78b | *Hafnia sp. CBA7124* | AP017469 |
| 79a | *Serratia liquefaciens strain AL117* | MG819252 |
| 80a | *Hafnia alvei strain CBA7135* | CP021971 |
| 81a | *Kluyvera cryocrescens* | LC060917 |
| 81b | *Serratia liquefaciens strain RHMR 21* | MF662820 |
| 82a | *Aeromonas salmonicida subsp. achromogenes strain HLD01* | MH478113 |

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|---|---|---|
| 82b | *Morganella morganii subsp. sibonii strain 3522-75* | HM122053 |
| 83a | *Hafnia alvei strain CBA7135* | CP021971 |
| 84a | *Serratia liquefaciens strain FC6859* | MH497592 |
| 84b | *Erwinia persicina strain P* | MH362699 |
| 85a | *Morganella morganii subsp. sibonii strain 3522-75* | HM122053 |
| 86a | *Aeromonas sp. AKB-2008-HE79* | AM989244 |
| 86b | *Serratia liquefaciens ATCC 27592* | CP006253 |
| 88a | *Serratia liquefaciens strain RHMR 21* | MF662820 |
| 89a | *Serratia liquefaciens strain LZ-24* | KU950364 |
| 89b | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 90a | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 91a | *Hafnia alvei strain CBA7135* | CP021971 |
| 92a | *Hafnia alvei strain CBA7135* | CP021971 |
| 94a | *Serratia liquefaciens strain AL117* | MG819252 |
| 94b | *Hafnia sp. CBA7124* | AP017469 |
| 95a | *Morganella morganii subsp. sibonii strain 3522-75* | HM122053 |
| 96a | *Morganella morganii subsp. sibonii strain 3522-75* | HM122053 |
| 96b | *Serratia sp. HX-B01* | KF501474 |
| 97a | *Buttiauxella izardii strain PgBe218* | MH211307 |
| 98a | *Hafnia alvei strain FC2951* | MH532496 |
| 98b | *Aeromonas sobria JCM 2366* | LC383907 |
| 99a | *Aeromonas sp. strain L23* | MH381782 |
| 100a | *Aeromonas salmonicida subsp. achromogenes strain HLD01* | MH478113 |
| 101a | *Hafnia alvei strain CBA7135* | CP021971 |
| 103a | *Hafnia alvei strain CBA7135* | CP021971 |
| 104a | *Serratia liquefaciens strain LZ-24* | KU950364 |
| 104b | *Aeromonas salmonicida subsp. achromogenes strain HLD01* | MH478113 |
| 105a | *Aeromonas salmonicida subsp. achromogenes strain HLD01* | MH478113 |
| 106a | *Aeromonas salmonicida subsp. achromogenes strain HLD01* | MH478113 |
| 106b | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 107a | *Aeromonas salmonicida subsp. achromogenes strain HLD01* | MH478113 |

| | | |
|---|---|---|
| 107b | *Serratia liquefaciens strain FDAARGOS_125* | CP014017 |
| 108a | *Serratia liquefaciens strain B7* | KJ781948 |
| 108b | *Serratia liquefaciens strain FC6859* | MH497592 |
| 109a | *Obesumbacterium proteus strain AC10* | MG649998 |
| 110a | *Hafnia alvei strain FC2951* | MH532496 |
| 110b | *Pseudomonas protegens strain TPD3011* | MH221127 |
| 111b | *Serratia liquefaciens strain AL117* | MG819252 |
| 112a | *Citrobacter braakii strain HQ288930.1* | MH346234 |
| 113a | *Hafnia alvei strain CBA7135* | CP021971 |
| 113b | *Serratia plymuthica strain WCF42* | KF595075 |
| 114a | *Serratia liquefaciens strain AK-25* | KY863496 |
| 115a | *Pseudomonas protegens strain TPD3011* | MH221127 |
| 115b | *Lelliottia amnigena* | CP028520 |
| 116a | *Hafnia alvei strain FC2951* | MH532496 |
| 117a | *Serratia liquefaciens strain FC6859* | MH497592 |
| 118a | *Serratia fonticola strain FDAARGOS_411* | CP023956. |
| 118b | *Serratia liquefaciens strain LZ-24* | KU950364 |
| 119a | *Raoultella ornithinolytica strain FDAARGOS_431* | CP023888 |
| 119b | *Serratia liquefaciens strain RHMR 21* | MF662820 |
| 120a | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 121a | *Serratia sp. DT-1* | JQ954965 |
| 122b | *Aeromonas sp. strain L23* | MH381782 |
| 124a | *Aeromonas sp. strain L23* | MH381782 |
| 125a | *Serratia liquefaciens strain RHMR 21* | MF662820 |
| 126a | *Serratia sp. UIWRF1049* | KR189040 |
| 127a | *Pseudomonas protegens strain TPD3011* | MH221127 |
| 127b | *Hafnia alvei strain FC2951* | MH532496 |
| 128a | *Hafnia alvei strain CBA7135* | CP021971 |
| 129a | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 129b | *Serratia liquefaciens ATCC 27592* | CP006252 |
| 130a | *Hafnia paralvei strain FDAARGOS_158* | CP014031 |

| | | |
|---|---|---|
| 131a | *Serratia liquefaciens strain AL117* | MG819252 |
| 131b | *Serratia liquefaciens strain Noth_8* | MF716555 |
| 132a | *Hafnia sp. CBA7124* | AP017469 |
| 133a | *Serratia liquefaciens strain AL117* | MG819252 |
| 134a | *Hafnia alvei strain FC2951* | MH532496 |
| 135a | *Citrobacter freundii strain MRB0903* | GU126681 |
| 136a | *Aeromonas salmonicida subsp. achromogenes strain HLD01* | MH478113 |
| 136b | *Rahnella aquatilis strain PRE12* | MG966290 |
| 137a | *Hafnia alvei strain CBA7135* | CP021971 |
| 137b | *Microvirgula aerodenitrificans strain BE2.4* | CP028519 |
| 138a | *Pseudomonas sp. AKB-2008-HE71* | AM989287 |
| 138b | *Serratia liquefaciens strain FC6859* | MH497592 |
| 139b | *Aeromonas sobria JCM 2366* | LC383907 |
| 140a | *Serratia liquefaciens strain AL117* | MG819252 |
| 140b | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 141a | *Serratia sp. HX-B01* | KF501474 |
| 142a | *Hafnia alvei strain 14* | KY849243 |
| 142b | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 143a | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 143b | *Serratia fonticola strain FDAARGOS_411* | CP023956 |
| 144a | *Microvirgula aerodenitrificans strain BE2.4* | CP028519 |
| 144b | *Hafnia alvei strain CBA7135* | CP021971 |
| 145a | *Aeromonas salmonicida strain A527* | CP022550 |
| 145b | *Serratia liquefaciens strain B7* | KJ781948 |
| 146a | *Serratia liquefaciens strain RHMR 21* | MF662820 |
| 147a | *Serratia liquefaciens strain FC6859* | MH497592 |
| 147b | *Aeromonas salmonicida subsp. achromogenes strain HLD01* | MH478113 |
| 148a | *Raoultella ornithinolytica strain YSH-3* | MH185873 |
| 149a | *Aeromonas salmonicida subsp. achromogenes strain HLD01* | MH478113 |
| 150a | *Serratia liquefaciens strain FC6859* | MH497592 |
| 151a | *Serratia liquefaciens strain FC6859* | MH497592 |

| | | |
|---|---|---|
| 152a | *Hafnia alvei strain FC2951* | MH532496 |
| 153a | *Serratia liquefaciens strain HUMV-21* | CP011303 |
| 154a | *Enterobacter amnigenus strain S_T_MRS_17* | JX860617 |
| 155a | *Aeromonas sobria JCM 2366* | LC383907 |
| 156a | *Hafnia alvei strain FC2951* | MH532496 |
| 157a | *Aeromonas sp. strain L23* | MH381782 |
| 158a | *Hafnia alvei strain FC2951* | MH532496 |
| 159a | *Raoultella ornithinolytica strain FDAARGOS 431* | CP023888 |
| 160a | *Raoultella terrigena strain PG201007011702* | JN815233 |
| 161a | *Hafnia sp. CBA7124* | AP017469 |
| 162a | *Hafnia alvei strain FC2951* | MH532496 |
| 164a | *Shewanella seohaensis strain NR_108852.* | MH071535 |
| 165a | *Hafnia alvei strain FC2951* | MH532496 |
| 166a | *Hafnia alvei strain FC2951* | MH532496 |
| 167a | *Morganella morganii strain CU-BS1* | MF164158 |
| 168a | *Aeromonas sp. RA11* | FJ898302 |
| 168b | *Enterobacter sp. UIWRF0484* | KR189673 |
| 169a | *Serratia liquefaciens strain AK-25* | KY863496 |
| 170a | *Morganella sp. ESBL68B6_12EESBL* | KJ831422 |
| 171a | *Lelliottia nimipressuralis strain BB2.2* | MH681482 |
| 171b | *Serratia liquefaciens strain Sneb2480* | MG132665 |
| 173a | *Serratia liquefaciens strain FC6859* | MH497592 |
| 173b | *Citrobacter freundii complex sp. CFNIH3* | CP026235 |
| 174a | *Microvirgula aerodenitrificans strain WB22* | MH196456 |
| 175a | *Aeromonas sp. strain L23* | MH381782 |
| 176a | *Serratia liquefaciens strain FC6859* | MH497592 |
| 177a | *Hafnia alvei strain FC2951* | MH532496 |
| 178a | *Stenotrophomonas maltophilia strain RS17* | MF536870 |
| 179a | *Serratia sp. DT-1* | JQ954965 |
| 180a | *Raoultella planticola strain FDAARGOS_64* | CP026047 |
| 182a | *Raoultella planticola strain FDAARGOS_64* | CP026047 |

| | | |
|---|---|---|
| 183a | *Proteus sp. strain ATCC 51469* | MG269476 |
| 185a | *Aeromonas encheleia strain CECT4342* | NR_118042 |
| 186a | *Hafnia alvei strain CBA7135* | CP021971 |
| 186b | *Hafnia sp. CBA7124* | AP017469 |
| 188a | *Serratia liquefaciens strain AL117* | MG819252 |
| 189a | *Serratia liquefaciens strain FDAARGOS 125* | CP014017 |
| 190a | *Serratia liquefaciens strain FC6859* | MH497592 |
| 191a | *Serratia liquefaciens strain FC6859* | MH497592 |
| 192a | *Buttiauxella izardii strain PgBe218* | MH211307 |
| 192b | *Serratia liquefaciens strain FC6859* | MH497592 |
| 193a | *Erwinia amylovora strain P3* | MF777033 |
| 194a | *Citrobacter braakii strain FC2965* | MH532470 |
| 195a | *Serratia liquefaciens strain FC6859* | MH497592 |
| 196a | *Aeromonas veronii strain CYJ205* | FJ940849 |
| 197a | *Citrobacter braakii strain FC2965* | MH532470 |
| 199a | *Serratia sp. strain 4M4* | KY611735 |
| 200a | *Serratia fonticola strain FDAARGOS_411* | CP023956 |

## 4.4 DOMINANT ENTEROBACTERICEAE SPECIES IN VEGETABLES

*Serratia* species were the most dominant Enterobacteriaceae in all vegetable types sampled in the Johannesburg Metropolis. This was followed by *Hafnia* species, which were present in all vegetable samples except for broccoli. *Aeromonas* species were the most dominant bacteria in broccoli, followed by cauliflower (Figure 4.4).

In terms of the distribution of the four most dominant Enterobacteriaceae in different vegetable types, spinach (24%) and cabbages (19%) were the most dominant leafy vegetables from which *Aeromonas* species were isolated. While chomolia (27%) and spinach (25%) were the most dominant leafy vegetable from which *Hafnia* species were isolated. Furthermore, Lettuce (55%)

and spinach (14%) were the most dominant leafy vegetables from which *Pseudomonas* species were isolated, while chomolia (23%), lettuce (17%), and cabbage (17%) were the most dominant leafy vegetables from which *Serratia* species were isolated (Figure 4.5).

The genus *Serratia* (35%) was the most dominant with the most dominant of all isolates being *Serratia liquefaciens* (60.5%). The *Hafnia* genus (21%) had *Hafnia alvei* as the most dominant species, followed by *Aeromonas* (17%), with *Aeromonas salmonicida* (38.5%) being the most dominant. In the genus *Pseudomonas* (5%), the most dominant species was *Pseudomonas protegens* (Table 4.6).

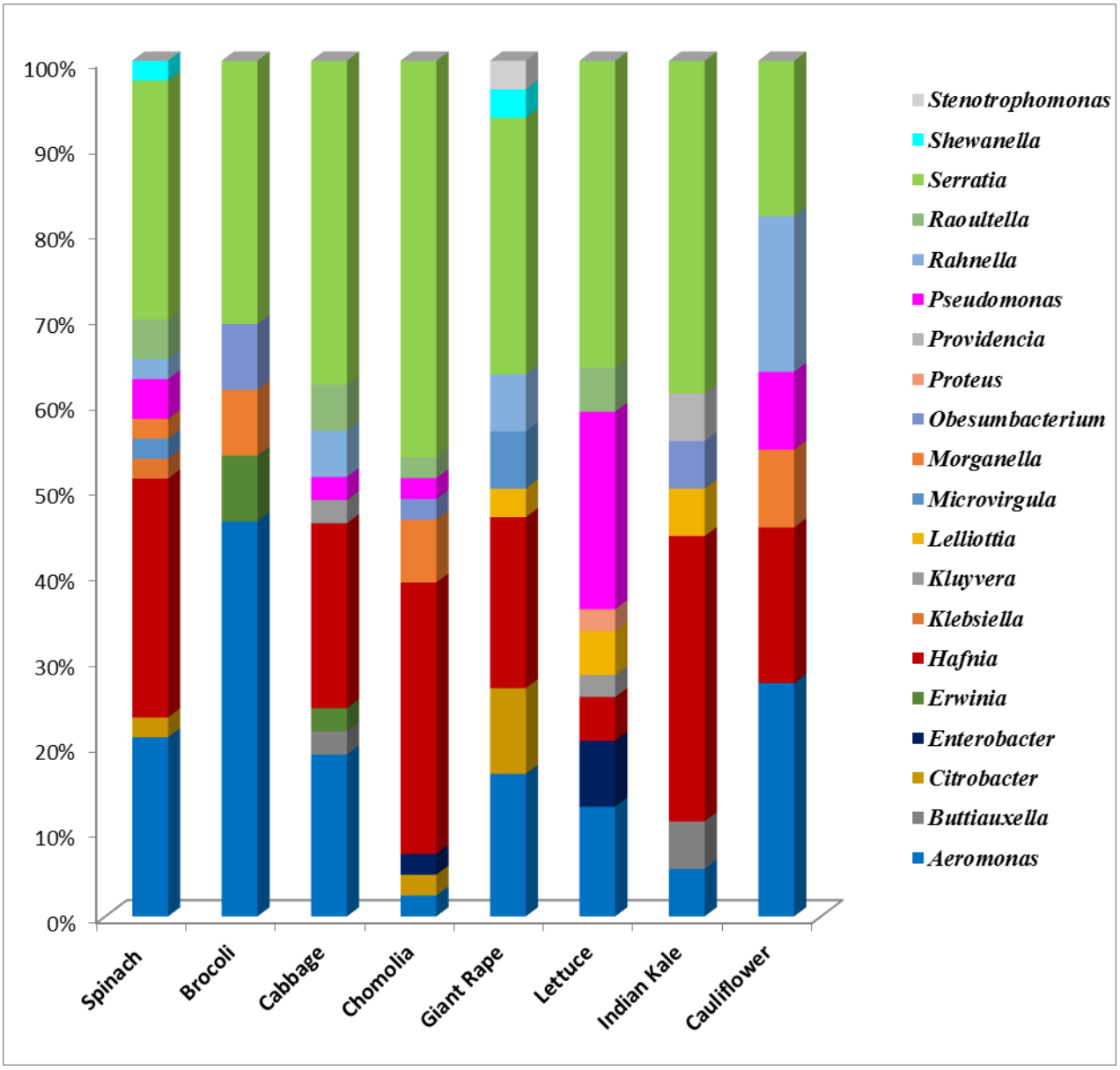


**Figure 4. 4: The percentage distribution of the Enterobacteriaceae genus in vegetables isolated sampled from markets in Johannesburg Metropolis.**

NB: The Y-axis values were calculated by expressing the number of isolates of a specific bacteria species, as a percentage of the total bacterial species isolated from each vegetable.

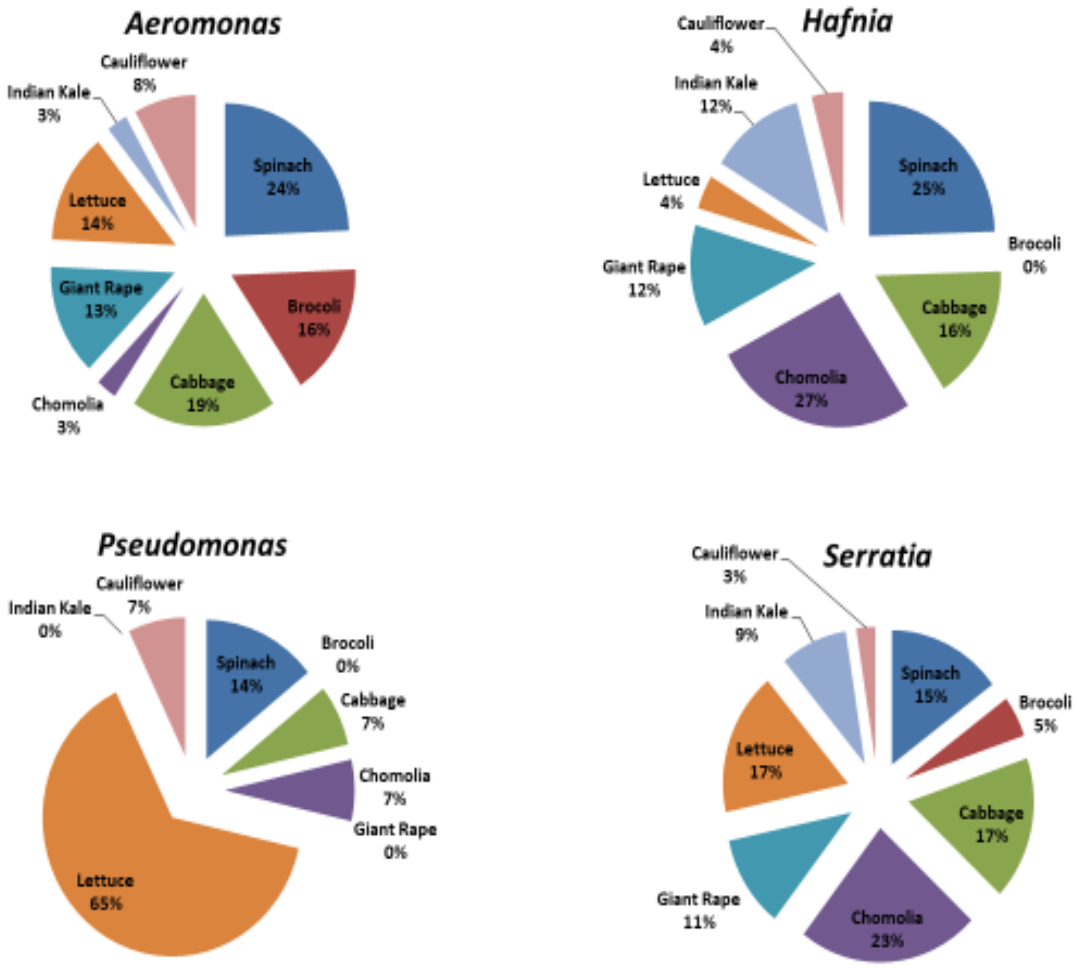


**Figure 4. 5: The percentage distribution of four of the most dominant Enterobacteriaceae genus (*Aeromonas, Hafnia, Pseudomonas* and *Serratia*) in different types of leafy vegetables sampled from markets in the Johannesburg Metropolis.**

**Table 4.6: The percentage distribution of species of the dominant Enterobacteriaceae genus in leafy vegetables sampled from markets in the Johannesburg Metropolis**

| Genus (%), N = 232 | Dominant species (%), N = 39 |
|---|---|
| *Aeromonas* (17%) | *Aeromonas veronii* (2.6%) |
| | *Aeromonas encheleia* (2.6%) |
| | *Aeromonas hydrophila* (5.1%) |
| | *Aeromonas salmonicida* (38.5%) |
| | *Aeromonas piscola* (5.1%) |
| | *Aeromonas sobria* (15.4%) |
| *Hafnia* (21%) | **Dominant species (%), N = 49** |
| | *Hafnia alvei* (71.4%) |
| | *Hafnia alvei* (71.4%) |
| | *Hafnia paralvei* (12.2%) |
| | *Hafnia sp. CBA7124* (12.2%) |
| *Pseudomonas* (5%) | **Dominant species (%), N = 12** |
| | *Pseudomonas protegens* (50%) |
| | *Pseudomonas japonina* (8.3%) |
| | *Pseudomonas putida* (8.3%) |
| | *Pseudomonas rhodesiae* (25%) |
| *Serratia* (35%) | **Dominant species (%), N = 81** |
| | *Serratia fanticola* (27.2%) |
| | *Serratia glossianae* (1.2%) |
| | *Serratia liquefaciens* (60.5%) |
| | *Serratia plymuthica* (2.5%) |
| | |

## 4.5 DOMINANT ENTEROBACTERIACEAE SPECIES IN VEGETABLES FROM DIFFERENT REGIONS

*Serratia*, *Hafnia*, and *Aeromonas* were the most dominant Enterobacteriaceae species isolated from vegetables from all the five regions in the Johannesburg Metropolis, followed by *Pseudomonas* which was present in all the regions, except Roodepoort (Figure 4.6).

In terms of the distribution of the four most dominant Enterobacteriaceae in different regions, JHB CBD (38%), Soweto (19%), and Yeoville (19%) were the most dominant regions from which *Aeromonas* species were isolated from vegetables, while JHB CBD (29%) and Yeoville (23%) were the most dominant regions from which *Hafnia* species were isolated from vegetables.

Furthermore, JHB CBD (24%) and Yeoville (23%) were the most dominant regions from which *Serratia* species were isolated, while Soweto (36%) and Yeoville (29%) were the most dominant regions from which *Pseudomonas* species were isolated from vegetables (Figure 4.7).

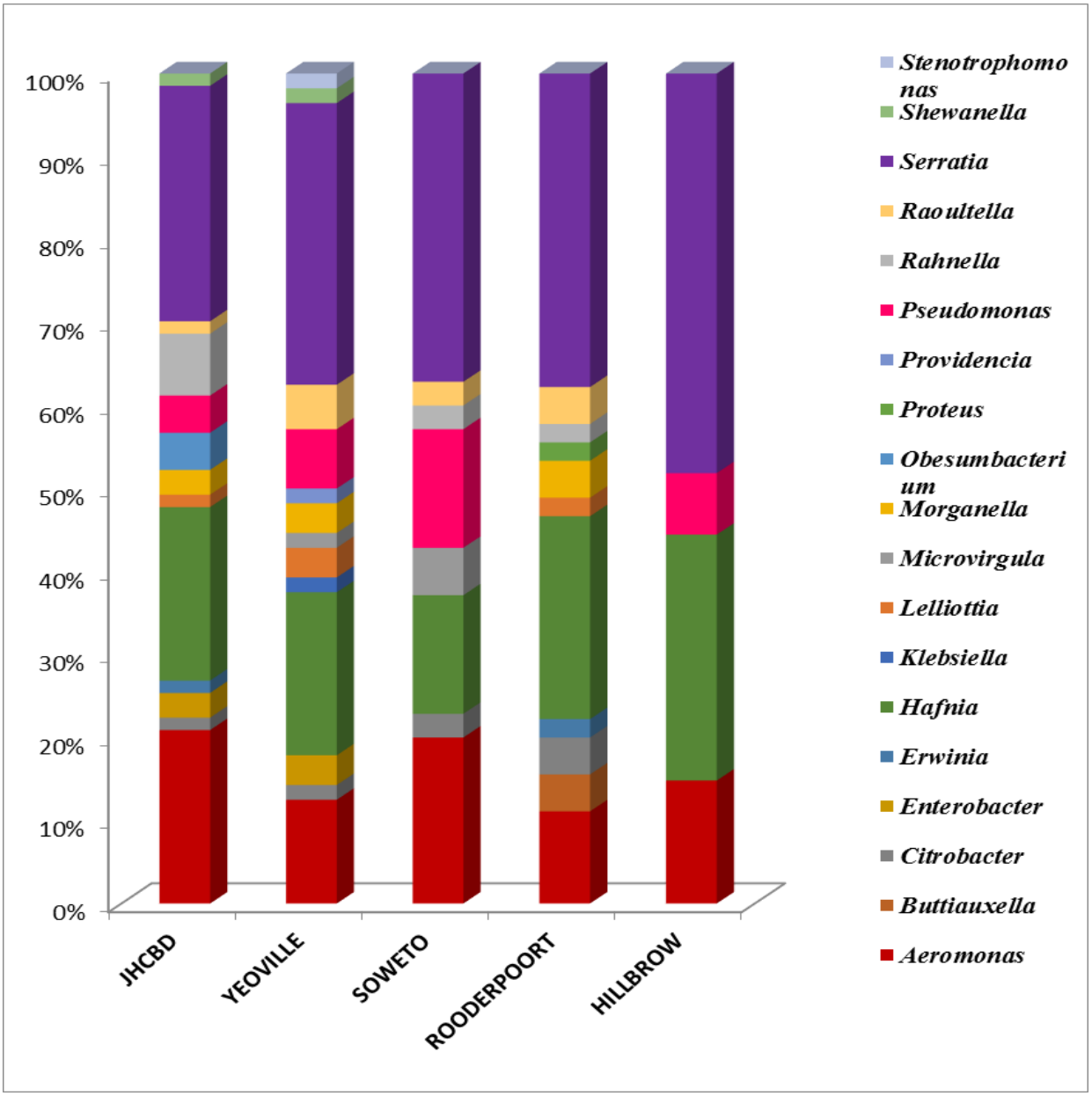


**Figure 4. 6: The distribution of the Enterobacteriaceae genus isolated from vegetables from different regions in the Johannesburg Metropolis.**

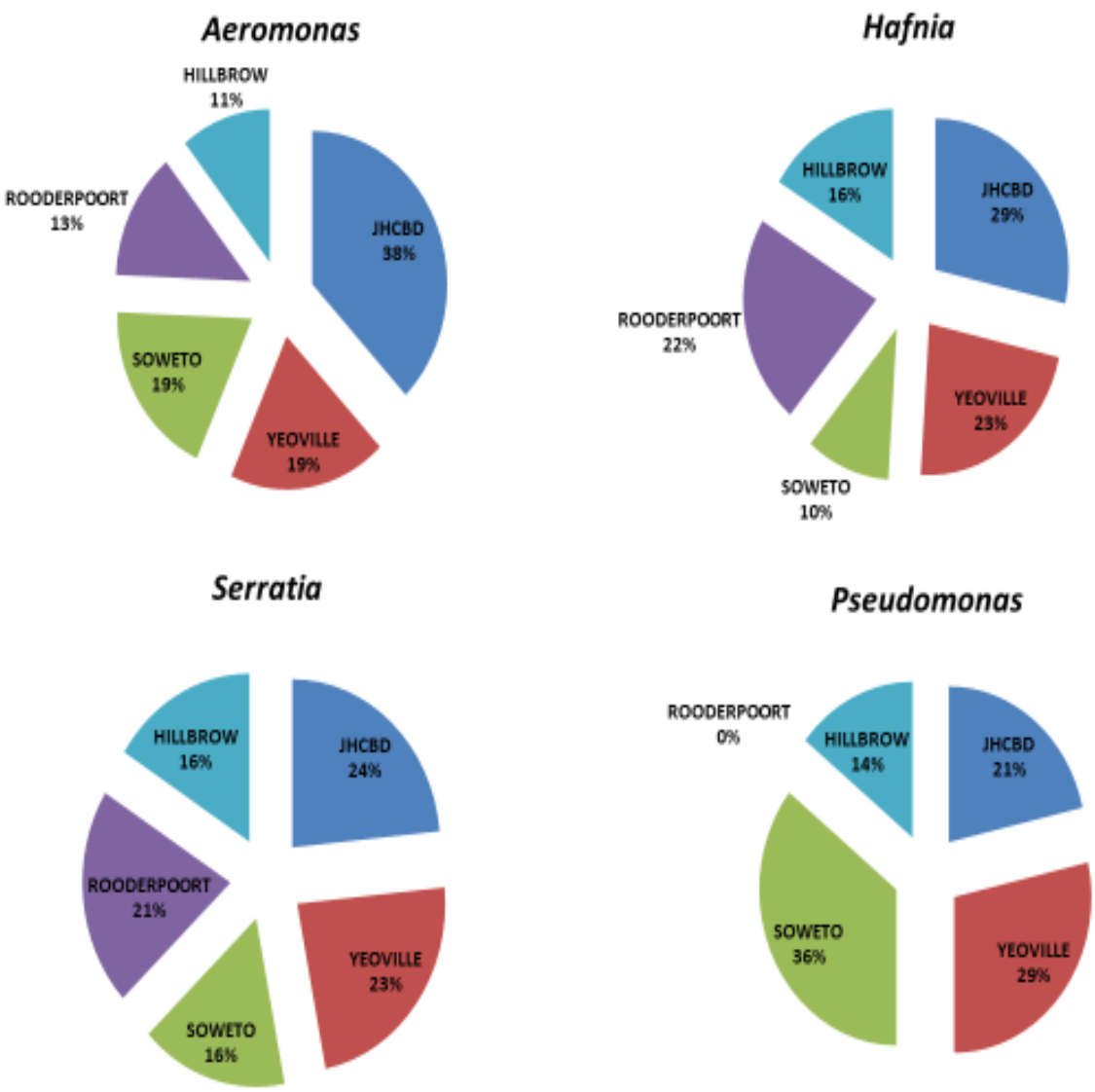


**Figure 4. 7: The percentage distribution of the four most dominant Enterobacteriaceae genus (*Aeromonas*, *Hafnia*, *Pseudomonas* and *Serratia*) collected from the different regions in the Johannesburg Metropolis.**

## 4.6 DIVERSITY AND PHYLOGENETIC RELATIONSHIP OF ENTEROBACTERIACEAE SPECIES ISOLATED FROM DIFFERENT TYPES OF VEGETABLES

The Enterobacteriaceae species isolates from broccoli were divided into two main dominant subphyla with the *Aeromonas* genus being the most genetically distant. The other phylum consisted of a very closely genetically related *Morganella*, *Hafnia*, *Erwinia*, and *Serratia* genera. *Serratia liquefaciens* was the most diverse species and the most distant from *Aeromonas* species (Figure 4.8). The Enterobacteriaceae species isolates from cabbage were divided into five main dominant subphyla with the *Pseudomonas*, followed by *Aeromonas* genera being the most genetically distant. The other phylum consisted of genetically closely related genera including *Rahnella*, *Serratia*, *Enterobacter*, and *Hafnia*. *Hafnia alvei* was the most diverse species and the most distant from the *Pseudomonas* and *Aeromonas* species (Figure 4.9).

The Enterobacteriaceae species isolates form cauliflower were divided into three main subphyla consisting of *Pseudomonas* and *Aeromonas* genera, and lastly a group of closely genetically related genera consisting of *Morganella, Hafnia,* and *Serratia*. The most genetically distant genera were *Aeromonas* and *Pseudomonas* (Figure 4.10).

The Enterobacteriaceae species isolates from chomolia were divided into three main dominant subphyla consisting of the *Pseudomonas* and *Shewanella* genera and lastly a group of closely genetically related general consisting of the *Morganella*, *Raoultella*, *Kluyvera*, *Citrobacter*, *Hafnia*, and *Serratia* genera. The most genetically distant genus was *Pseudomonas* followed by *Sherwanella* (Figure 4.11). The Enterobacteriaceae species isolates from the giant English rape were divided into three main subphyla with *Microvirgula* followed by *Pseudomonas*, and lastly a

subphylum which consists of genetically closely related *Aeromonas*, *Serratia*, *Rahnella*, and *Hafnia* genera. *Serratia* and *Hafnia* species were the most genetically diverse. The most genetically distant genus was *Microvirgula* followed by *Pseudomonas* (Figure 4.12).

The Enterobacteriaceae species isolates from Indian kale were divided into three dominant subphyla consisting of *Aeromonas* followed by *Providencia*, and lastly a subphylum consisting mostly of genetically closely related *Hafnia* and *Serratia* genera. *Serratia* and *Hafnia* species were the most genetically diverse. The most genetically distant genera were *Aeromonas* followed by *Providencia* (Figure 4.13). The Enterobacteriaceae species isolates from lettuce were divided into three main dominant subphyla consisting of the *Pseudomonas* and *Aeromonas* genera, and lastly a subphylum consisting mostly of genetically closely related *Serratia* and *Hafnia* genera. *Serratia* and *Hafnia* species were the most genetically diverse. *Pseudomonas* followed by *Aeromonas* were the most genetically distant genus (Figure 4.14). The Enterobacteriaceae species isolates from spinach were divided into four main dominant subphyla consisting of *Pseudomonas* and *Microvirgula* genera and lastly a subphylum consisting mostly of genetically closely related *Serratia* and *Hafnia* genera. *Serratia* and *Hafnia* species were the most genetically diverse. *Microvirgula* followed by *Pseudomonas* were the most genetically distant genus (Figure 4.15).

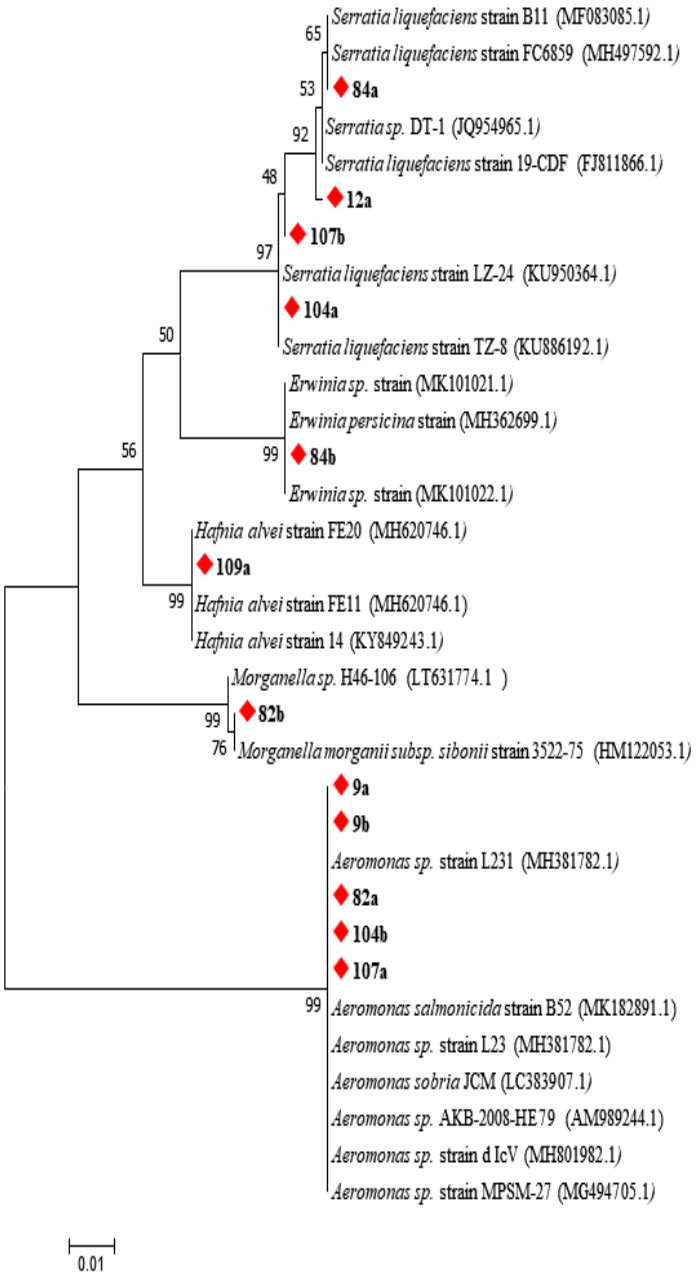


**Figure 4. 8: Phylogenetic analysis of Enterobacteriaceae species isolated from broccoli sold at informal markets in the Johannesburg Metropolis.**

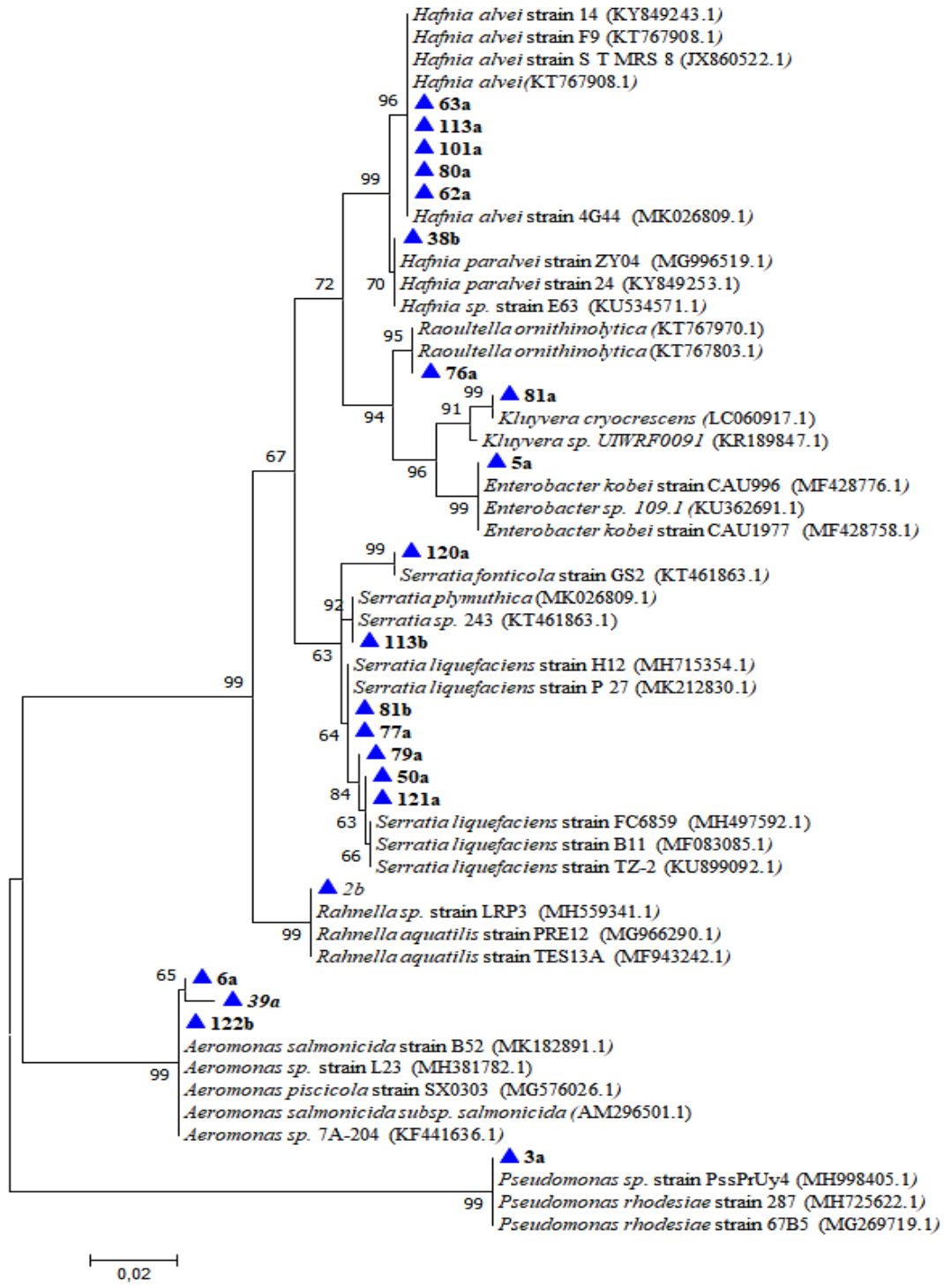


**Figure 4. 9: Phylogenetic analysis of Enterobacteriaceae species isolated from cabbage sold at informal markets in the Johannesburg Metropolis.**

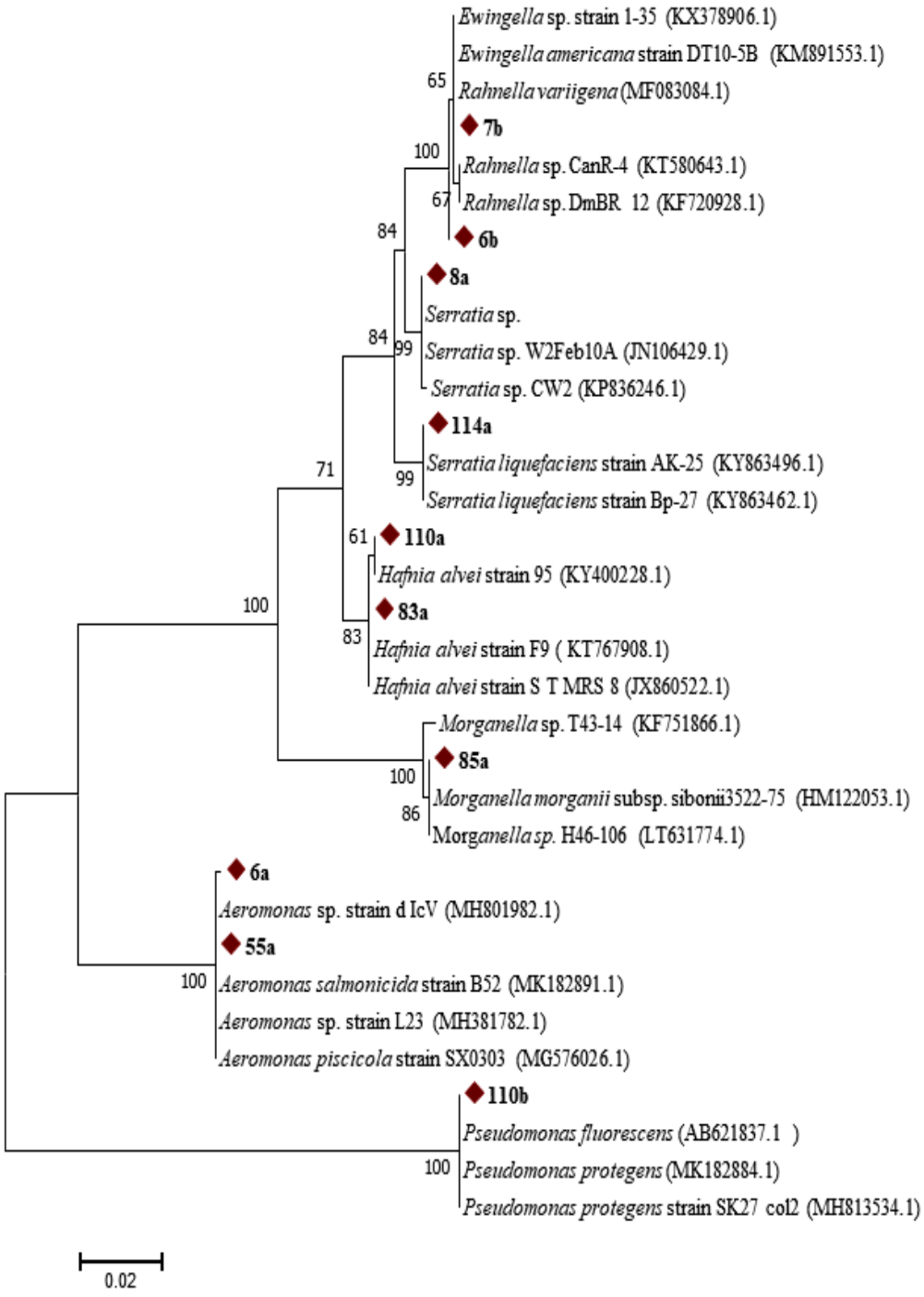


**Figure 4. 10: Phylogenetic analysis of Enterobacteriaceae species isolated from cauliflower sold at informal markets in the Johannesburg Metropolis.**

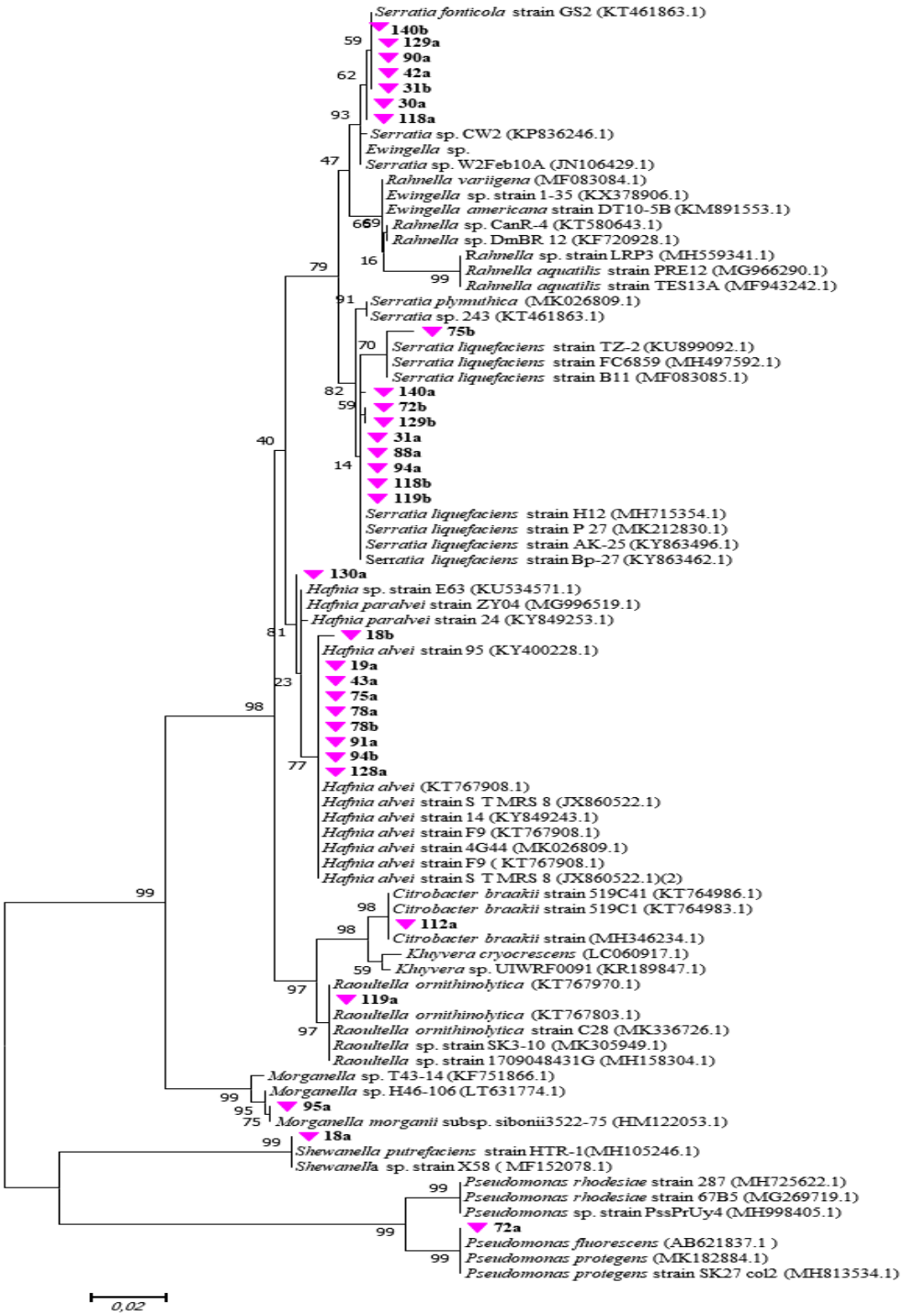


**Figure 4. 11: Phylogenetic analysis of Enterobacteriaceae species isolated from chomolia sold at informal markets in the Johannesburg Metropolis.**

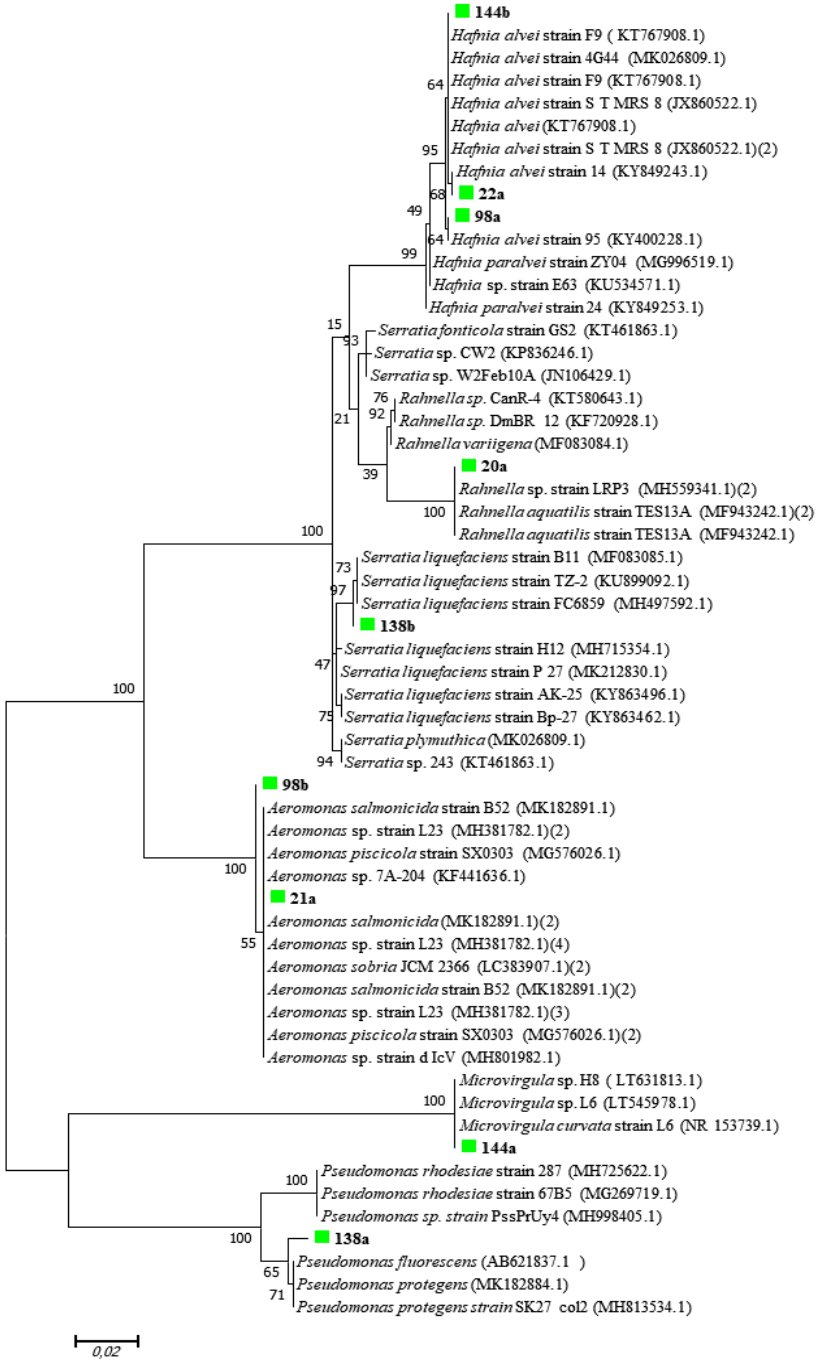


**Figure 4. 12: Phylogenetic analysis of Enterobacteriaceae species isolated from giant English rape sold at informal markets in the Johannesburg Metropolis.**

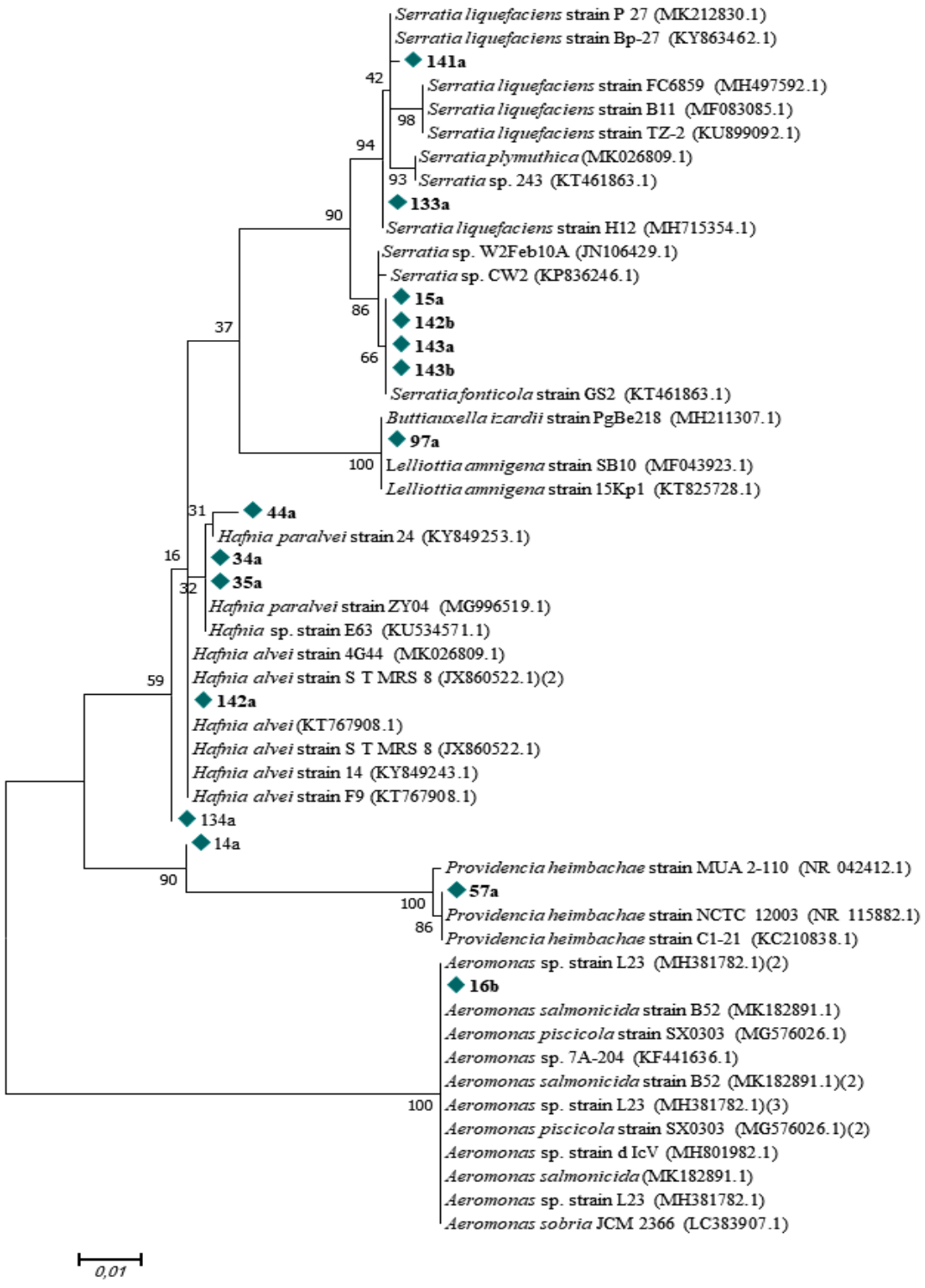


**Figure 4. 13: Phylogenetic analysis of Enterobacteriaceae species isolated from Indian Kale sold at informal markets in the Johannesburg Metropolis.**

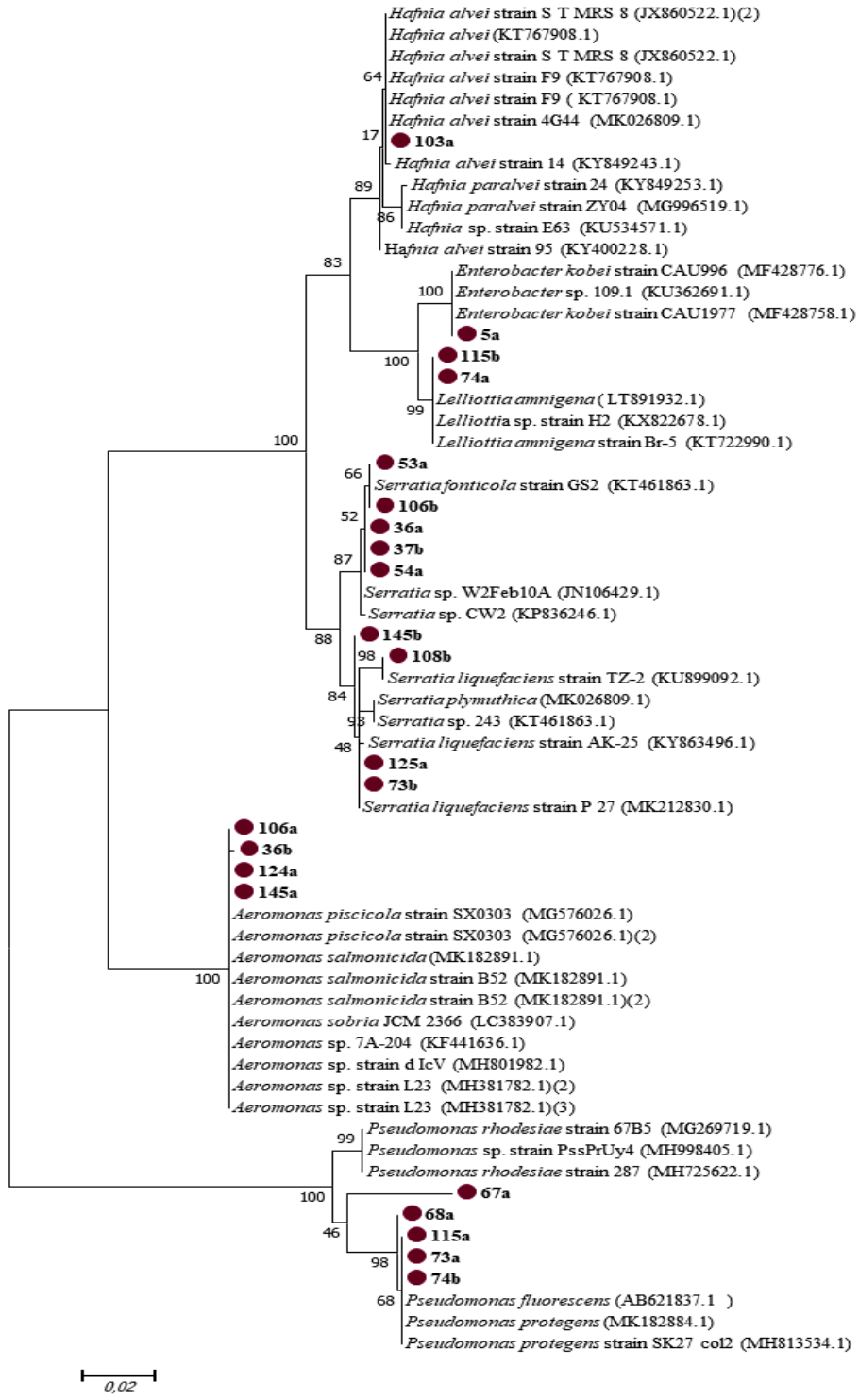


**Figure 4. 14: Phylogenetic analysis of Enterobacteriaceae species isolated from lettuce sold at informal markets in the Johannesburg Metropolis.**

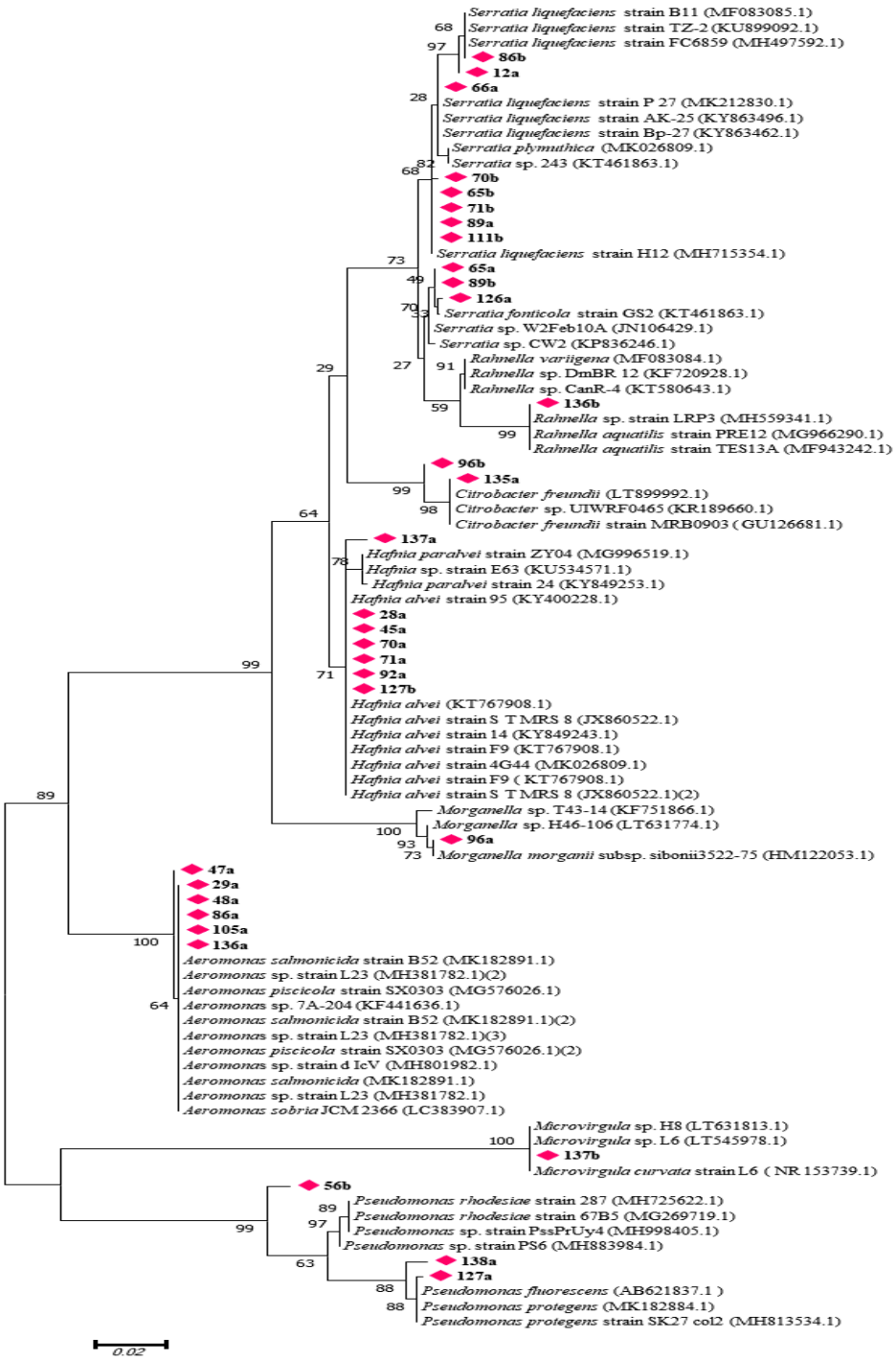


**Figure 4. 15: Phylogenetic analysis of Enterobacteriaceae species isolated from spinach sold at informal markets in the Johannesburg Metropolis.**

# CHAPTER 5:

# DISCUSSION

## 5.1 BACTERIAL QUALITY OF VEGETABLES

All the 201 vegetable samples collected from markets from the Johannesburg Metropolis had aerobic bacterial growth. The reason for this is cross-contamination from other vegetables and poor hygiene practices of vegetable handlers at the different markets (Desiree, 2019) and also airborne (Xu et al, 2018). The results show that the mean aerobic plate count of vegetables sold in the Johannesburg CBD and Hillbrow regions are significantly ($p \leq 0.05$) different from those from Yeoville and Soweto, while the vegetables from Roodepoort is not significantly different from those of the other regions. The differences in aerobic plate counts can be attributed to contamination from untreated irrigation water or different handling and storage procedures of the vegetables at different regions in the Johannesburg Metropolis (Buyukunal *et al.*, 2015). The aerobic colony count of different leafy vegetable types sold the Johannesburg Metropolis were not significantly different ($p > 0.05$). This could be due to contamination from irrigation water used in the production of the vegetables (Benti, Kebede and Menkir, 2014).

The aerobic growth count results from this study are much higher than that obtained from previous studies (Maffei, Silveira and Catanozi, 2013). The aerobic growth count is an indicator of hygiene quality of the vegetables. The significance of this is that the high aerobic growth count indicates poor vegetable quality, which may lead to a potential health risk to consumers (Zekar et al., 2017). From by observations and chats with the vendors, they used public toilets which did not have tap water for them to wash their hands. The significance of this is that

inadequate handwashing by vegetable handlers can increase contamination (Guangsu Xu, 2018; Robinson et al., 2017). Previous studies show that handwashing is important and it can reduce cross-contamination (Ataee et al., 2017). It was also observed that, to keep the vegetables moist, the vendors dip various vegetables in the same bucket of water which they will have collected from burst pipes or brought from their homes. This is a very unhygienic practice which might be one of the major contributors to cross-contamination (Murray et al., 2017), since bacteria can be transmitted from the contaminated water to the vegetables (Kumar and Kumar, 2017). The increased probability of cross-contamination could be the reason why there is no significant difference in the mean aerobic plate counts of different vegetables (Alemu et al., 2018).

## 5.2 ENTEROBACTERIACEAE QUALITY OF VEGETABLES

The Enterobacteriaceae counts of vegetables from each of the five regions in the Johannesburg Metropolis were not significantly ($p \leq 0.05$) different. The reason for this is that the vegetables were probably produced using irrigation water which has previously been found to have a potential source of pathogenic contamination (Akinde et al, 2016). The high Enterobacteriaceae counts could be due to poor hygienic conditions. The food handlers' hands have high prevalence of Enterobacteriaceae and if the proper washing procedure is not followed, the Enterobacteriaceae may be transmitted to the vegetables (Ntomola Sophia Swalehe, 2014), which results in unsatisfactory quality for consumers (Al-kharousi et al, 2016). Poor handling and storage at each sampling site were observed and this could result in cross contamination of vegetables (Lambrechts et al., 2014).

In a study exploring the good manufacturing practice and microbial contamination sources in orange-fleshed sweet potato puree, poor plant hygiene increased the contamination of the puree with Enterobacteriaceae and other microorganisms (Malavi et al, 2016). Enterobacteriaceae in hospitalised patients in a hospital in Ethiopia were transmitted from two infected children through poor hygiene of health workers to other paediatric patients, resulting in the spread of the infection (Desta et al, 2016).

## 5.3 PREDOMINANT ENTEROBACTERIACEAE SPECIES IN DIFFERENT VEGETABLES TYPES

This study shows that spinach (24%) and cabbage (19%) were the most dominant leafy vegetables from which *Aeromonas* species were isolated. The reason for this is that the *Aeromonas* species from the environment attach themselves onto the surface of vegetables, forming biofilms and resulting in their dominance. (Elhariry, 2015). *Aeromonas* species have previously been isolated from cabbage, spinach, and other vegetables from Punjab, India (Kamalpreet et al, 2017). *Aeromonas* species was identified as opportunistic species which was a causative agent of endophthalmitis in a 55-year old patient (Varshney et al., 2018) and can therefore, be a health risk to consumers.

Chomolia (27%) and spinach (25%) were the most dominant leafy vegetable from which *Hafnia* species were isolated. The reason for this is the type of farming practices used for the production of these vegetables (Merlini et al., 2018). Lettuce (55%) and spinach (14%) were the most dominant leafy vegetables from which *Pseudomonas* species were isolated, possibly due to contamination from irrigation water or the soil where the vegetables were grown (Alam et al.,

2015). *Pseudomonas* species were part of the pathogenic bacteria isolated from both irrigation water and vegetables in South West Nigeria, which indicated contamination of the vegetables was increased due to the use of contaminated irrigation water (Akinde et al, 2016).

Chomolia (23%), lettuce (17%), and cabbage (17%) were the most dominant leafy vegetables from which *Serratia* species were isolated. The reason for this is poor hygiene practices, as previously show in transmission of *Serratia* species from hospital personnel to patients (Ikumapayi et al., 2016). *Serratia* species are a health risk to humans as they can cause nosocomial infections of the respiratory and urinary tracts (Yeung et al., 2018). *Serratia* species are opportunistic pathogens which were isolated from an immunocompetent patient but had uncontrolled diabetes, resulting in the patient suffering from severe osteomyelitis and septic arthritis (Hadid et al, 2015). This is not the first time *Serratia* species have been isolated from vegetables. Previously, these species have been isolated from retail vegetables such as lettuce and radish sold in retail markets in Netherlands (Hoek et al, 2015). *Pseudomonas* species are one of the most dominant isolates from vegetables with 55% isolated from lettuce, possibly due to poor irrigation water quality (Akinde et al., 2016). These results are in line with previous studies which indicated that one of the Enterobacteriaceae species transmitted by vegetables, such as lettuce, is the carbapenem-resistant *Pseudomonas aeruginosa* (Holzel et al, 2018).

## 5.4 PREDOMINANT ENTEROBACTERIACEAE SPECIES IN VEGETABLES FROM DIFFERENT REGIONS

*Serratia* (45%), *Hafnia* (27%), and *Aeromonas* (20%) species were the most dominant Enterobacteriaceae isolated from leafy vegetables in all five regions in the Johannesburg Metropolis. This is due to poor handling of vegetables in the informal markets which could result in cross-contamination from hands of vendors to the displayed vegetables (Nipa et al., 2011). The high prevalence of these species may have been due to pre-harvest contamination from untreated/contaminated irrigation water (Akinde et al., 2016). Enterobacterial contamination of lettuce can occur through contaminated irrigation water and can continue over a long period of time (Oliveira et al, 2012).

## 5.5 DIVERSITY OF ENTEROBACTERIACEAE SPECIES ISOLATED FROM DIFFERENT TYPES OF VEGETABLES

In this study, the results show that the most genetically diverse Enterobacteriaceae communities were those isolated from cabbage, chomolia, and giant English rape. The reason for this is that the vegetables might have been exposed to different sources of contamination such as poor hygiene (Lehto et al., 2011), contaminated irrigation water (Akinde et al., 2016), and the use of contaminated organic fertilisers (Szczech et al., 2018). Previous studies conducted in Boulder CO, USA on Enterobacteriaceae communities associated with surfaces of fresh fruits and vegetables showed that vegetables such as sprouts, spinach, lettuce, tomato, pepper, and strawberries had high abundance of Enterobacteriaceae, but the communities differed depending whether the farming process was convectional or organic (Leff et al, 2013).

The Enterobacteriaceae communities in giant English rape and spinach were similar, but they differed from the closely similar Enterobacteriaceae communities of cabbage, lettuce, and cauliflower. The leaf surface area of spinach and giant English rape has less grooves compared to that of cabbage, lettuce, and cauliflower. Bacteria easily attach to vegetable surfaces with more grooves than those with smooth surfaces (Warning and Datta, 2017). The cabbage had the most diverse Enterobacteriaceae with five different subphyla, followed by spinach with four. The reason for this is that cabbage has the largest surface area compared to spinach and this makes it easy for Enterobacteriaceae to attach onto the leaf surfaces (Alemu et al, 2018). A large surface area increases exposure to contamination, resulting in the formation of bacteria biofilm on vegetable surfaces (Kyere et al, 2019).

# CHAPTER 6:

# CONCLUSION AND RECOMMENDATIONS

## 6.1 CONCLUSION

This study shows that the vegetables sold at the informal markets in the Johannesburg Metropolis have high aerobic growth counts which are not significantly different across all the eight vegetables tested. However, the aerobic growth counts of vegetables from Johannesburg CBD and Hillbrow were significantly lower than those of Yeoville and Soweto. The high aerobic growth count is an indicator of poor hygiene.

The study results also indicate that the Enterobacteriaceae counts of different vegetables sold at the informal markets in different regions in the Johannesburg Metropolis are not significantly different. The dominant Enterobacteriaceae genera isolated form all the regions in the Johannesburg Metropolis were *Aeromonas*, *Hafnia*, and *Serratia*, as well as *Pseudomonas*. These bacteria are opportunistic pathogens which may be a health risk to immunosuppressed consumers in the Johannesburg Metropolis.

Furthermore, the results from this study demonstrate that some Enterobacteriaceae communities in vegetables sold in Johannesburg Metropolis are diverse and can be a health risk to consumers. The knowledge of composition and diversity of Enterobacteriaceae communities in these vegetables may be useful in the establishment of measures to control contamination of vegetables sold to reduce transmission of pathogens to consumers in the Johannesburg Metropolis.

## 6.2 RECOMMENDATIONS

The researcher recommends that the informal market retailers regularly wash their hands and avoid washing various vegetables in the same bucket of water, as a precaution to reduce chances of cross-contamination.

In addition, the researcher recommends that the Johannesburg Metropolis improve the sanitation facilities and resources by ensuring readily available running water in the public restrooms used by vendors to reduce transmission of bacteria from unwashed hands to vegetables.

It is also recommended that hygiene awareness programmes be conducted by the Johannesburg Metropolis, Department of Health to educate both vendors and consumers on safe and hygienic food handling practices.

## REFERENCES


Akinde, S. B., Sunday, A. A., Adeyemi, F. M., Fakayode, I. B., Oluwajide, O. O., Adebunmi, A. A., Oloke, J. K. and Adebooye, C. O. (2016) 'Microbes in Irrigation Water and Fresh Vegetables : Potential Pathogenic Bacteria Assessment and Implications for Food Safety', *Journal of ABSA International*, 21(2), pp. 89–97. doi: 10.1177/1535676016652231.

Al-kharousi, Z. S., Guizani, N., Al-sadi, A. M., Al-bulushi, I. M. and Shaharoona, B. (2016) 'Hiding in Fresh Fruits and Vegetables : Opportunistic Pathogens May Cross Geographical Barriers', *International Journal of Microbiology*, 2016(May 2008), pp. 1–17.

Alam, S., Feroz, F., Rahman, H., Kanta D., K. and Noor, R. (2015) 'Microbiological contamination sources of freshly cultivated vegetables', *Nutrition & Food Science*, 45(4), pp. 646–658. doi: 10.1108/NFS-04-2015-0032.

Alemu, G., Mama, M. and Siraj, M. (2018) 'Bacterial contamination of vegetables sold in Arba Minch Town , Southern Ethiopia', *BMC Research Notes*. BioMed Central, 11, pp. 1–6. doi: 10.1186/s13104-018-3889-1.

Amrutha, B., Sundar, K. & Shetty, P.H. 2017, "Study on E. coli and Salmonella biofilms from fresh fruits and vegetables", Journal of Food Science and Technology, vol. 54, no. 5, pp. 1091-1097.

Ataee, R. A., Ataee, M. H., Tavana, A. M. and Salesi, M. (2017) 'Bacteriological Aspects of Hand Washing : A Key for Health Promotion and Infections Control', *International Journal of Preventive Medicine*, 8(16), pp. 1–10. doi: 10.4103/2008-7802.201923.

Benti, G., Kebede, A. and Menkir, S. (2014) 'Assessment of bacteriological contaminants of some vegetables irrigated with Awash River water in selected farms around Adama town , Ethiopia', *Journal of Microbiology and Antimicrobials*, 6(February), pp. 37–42. doi: 10.5897/JMA2013.0275.

Buyukunal, S. K., Issa, G., Aksu, F. and Vural, A. (2015) 'Microbiological Quality of Fresh Vegetables and Fruits Collected from Supermarkets in Istanbul , Turkey', *Journal of Food and Nutrition Sciences*, 3(4), pp. 152–159. doi: 10.11648/j.jfns.20150304.13.

Campos, J., Mourão, J., Pestana, N., Peixe, L., Novais, C. and Antunes, P. (2013) 'Microbiology Microbiological quality of ready-to-eat salads : An underestimated vehicle of bacteria and clinically relevant antibiotic resistance genes', *International Journal of Food Microbiology*. Elsevier B.V., 166(3), pp. 464–470. doi: 10.1016/j.ijfoodmicro.2013.08.005.

Castro-Ibanez, I., Gil, M. I. and Allende, A. (2017) 'LWT - Food Science and Technology Ready-to-eat vegetables : Current problems and potential solutions to reduce microbial risk in the production chain n', 85, pp. 284–292. doi: 10.1016/j.lwt.2016.11.073.

Chikara, S., Dalasanur, L., Singhal, J., Horne, D., Awasthi, S. and Singhal, S. S . (2018) 'Oxidative stress and dietary phytochemicals : Role in cancer chemoprevention and treatment', *Cancer Letters*. Elsevier Ltd, 413, pp. 122–134. doi: 10.1016/j.canlet.2017.11.002.

Desiree, K. (2019) *Characterizing food safety aspects of the Cambodian vegetable value-chain: A quantitative and qualitative investigation of biological hazards and food safety practices in Cambodia*. Kansas State University.

Douesset, X., Jaffres, E. and Zagorec, M. (2016) 'Spoilage : Bacterial Spoilage', *Encyclopedia of Food and Health*, pp. 106–112. doi: 10.1016/B978-0-12-384947-2.00649-8.

Elhariry, H. M. (2011) 'Biofilm Formation by Aeromonas hydrophila on Green-Leafy Vegetables : Cabbage and Lettuce', *Foodborne Pathogens And Disease*, 8(1), pp. 125–131. doi: 10.1089/fpd.2010.0642.

Hadid, H., Usman, M. and Thapa, S. (2015) 'Case Report Severe Osteomyelitis and Septic Arthritis due to Serratia marcescens in an Immunocompetent Patient', 2015, pp. 12–15.

Hoek, A. H. A. M. V., Veenman, C., Overbeek, W. M. V., Lynch, G., Maria, A., Husman, D. R. and Blaak, H., (2015) 'Prevalence and characterization of ESBL- and AmpC-producing Enterobacteriaceae on retail vegetables', *International Journal of Food Microbiology*. Elsevier B.V., 204, pp. 1–8. doi: 10.1016/j.ijfoodmicro.2015.03.014.

Holzel, C. S., Tetens, J. L. and Schwaiger, K. (2018) 'Unraveling the Role of Vegetables in Spreading Antimicrobial-Resistant Bacteria : A Need for Quantitative Risk Assessment', *Foodborne Pathogens And Disease*, 15(11). doi: 10.1089/fpd.2018.2501.

Igbeneghu, O. A. and Abdu, A. B. (2014) 'Multiple Antibiotic-resistant Bacteria on Fluted Pumpkin Leaves , a Herb of Therapeutic Value', 32(2), pp. 176–182.

Ikumapayi, U. N., Kanteh, A., Manneh, J., Lamin, M.and Mackenzie, G. A. (2016) 'An outbreak of Serratia liquefaciens at a rural health center in The Gambia', *The Journal of Infrection in Developing Countries*, 10. doi: 10.3855/jidc.7184.

Kumar, S. M. and Kumar, V. (2017) ‘A study on prevalence of microbial contamination on the surface of raw salad vegetables’, *3 Biotech*. Springer Berlin Heidelberg, 7(1), pp. 1–9. doi: 10.1007/s13205-016-0585-5.

Kyere, E. O.,Foong, G., Palmer, J., Wargent, J. J., Fletcher, G. C. and Flint, S. (2019) ‘Rapid attachment of Listeria monocytogenes to hydroponic and soil grown lettuce leaves’, Food Control. Elsevier, 101(January), pp. 77–80. doi: 10.1016/j.foodcont.2019.02.015.

Lambrechts, A., Human, I. S., Doughari, J. H. and Lues, J. F. R. (2014) ‘Bacterial contamination of the hands of food handlers as indicator of hand washing efficacy in some convenient food industries’, 30(4), pp. 755–758.

Leff, J. W. and Fierer, N. (2013) ‘Bacterial Communities Associated with the Surfaces of Fresh Fruits and Vegetables’, 8(3), pp. 1–10. doi: 10.1371/journal.pone.0059310.

Lehto, M., Kuisma, R., Määttä, J., Kymäläinen, H. and Mäki, M. (2011) ‘Hygienic level and surface contamination in fresh-cut vegetable production plants’, *Food Control*. Elsevier Ltd, 22(3–4), pp. 469–475. doi: 10.1016/j.foodcont.2010.09.029.

Liu, B., Zhang, X., Wan, S., Hao, J., Jiang, R. and Song, F. (2018) ‘Characteristics of Carbapenem-Resistant Enterobacteriaceae in Ready-to-Eat Vegetables in China’, *Frontiers in Microbiology*, 9(1147). doi: 10.3389/fmicb.2018.01147.

Maffei, D. F., Silveira, N. F. de A. and Catanozi, M. da P. L. M. (2013) ‘Microbiological quality of organic and conventional vegetables sold in Brazil’, *Food Control*. Elsevier Ltd, 29(1), pp. 226–230. doi: 10.1016/j.foodcont.2012.06.013.

Merlini, V. V., Pena, F. D., Thimoteo, D., Oliveira, J. M., Rostagno, M. A., Elisabete, A. and Antunes, C. (2018) ‘Microbiological Quality of Organic and Conventional Leafy Vegetables’, *Journal of Food Quality*, 2018, pp. 1–7. doi: https://doi.org/10.1155/2018/4908316.

Murray, K., Wu, F., Shi, J., Xue, S. J. and Warriner, K. (2017) ‘Challenges in the microbiological food safety of fresh produce : Limitations of post-harvest washing and the need for alternative interventions’, *Food Quality and Safety*, 1(December), pp. 289–301. doi: 10.1093/fqsafe/fyx027.

Nguyen-the, C. (2012) ‘LWT - Food Science and Technology Biological hazards in processed fruits and vegetables e Risk factors and impact of processing techniques’, *LWT - Food Science and Technology*. Elsevier Ltd, 49(2), pp. 172–177. doi: 10.1016/j.lwt.2012.07.002.

Nipa, M. N., Mazumdar, R. M., Islam, S., Bhuiyan, H. R. and Iqbal, A. (2011) 'Prevalence of Multi Drug Resistant Bacteria on Raw Salad Vegetables Sold in Major Markets of Chittagong City , Bangladesh', *Middle-East Journal of Scientific Research*, 10(1), pp. 70–77.

Reuland, E. A., Naiemi, N. and Raadsen, S. A. (2014) 'Prevalence of ESBL-producing Enterobacteriaceae in raw vegetables', pp. 1843–1846. doi: 10.1007/s10096-014-2142-7.

Robinson, A., Lee, H. J., Kwon, J., Cameron, E. and Todd, D. (2017) 'Adequate Hand Washing and Glove Use Are Necessary To Reduce Cross- Contamination from Hands with High Bacterial Loads Adequate Hand Washing and Glove Use Are Necessary To Reduce Cross-Contamination from Hands with High Bacterial Loads', *Journal of food protection*, 79(2), pp. 304–308. doi: 10.4315/0362-028X.JFP-15-342.

Snyder, A. B. and Worobo, R. W. (2018) 'The incidence and impact of microbial spoilage in the production of fruit and vegetable juices as reported by juice manufacturers', *Food Control*. Elsevier Ltd, 85, pp. 144–150. doi: 10.1016/j.foodcont.2017.09.025.

Ssemanda, N. J. (2017) 'Indicator microorganisms in fresh vegetables from " farm to fork " in Rwanda', *Food Control*. Elsevier Ltd, 75, pp. 126–133. doi: 10.1016/j.foodcont.2016.12.031.

Stephan, R., Althaus, D., Kiefer, S., Lehner, A., Hatz, C., Schmutz, C., Jost, M., Gerber, N., Mirjam, M., Baumgartner, A. and Herbert, H. (2015) 'Foodborne transmission of Listeria monocytogenes via ready-to-eat salad : A nationwide outbreak in Switzerland , 2013 e 2014', 57, pp. 2013–2016. doi: 10.1016/j.foodcont.2015.03.034.

Szczech, M.,Kowalska, B., Smoli, U., Maciorowski, R. and Michalska, A. (2018) 'International Journal of Food Microbiology Microbial quality of organic and conventional vegetables from Polish farms', 286(July), pp. 155–161. doi: 10.1016/j.ijfoodmicro.2018.08.018.

Tatsika, S., Karamanoli, K., Karayanni, H. and Genitsaris, S. (2019) 'Metagenomic Characterization of Bacterial Communities on Ready-to-Eat Vegetables and Effects of Household Washing on their Diversity and Composition', *Pathogens*, 8(37), pp. 1–18. doi: 10.3390/pathogens8010037.

Tien, Y., Li, B., Zhang, T., Scott, A., Murray, R., Sabourin, L., Marti, R. and Topp, E. (2017) 'Science of the Total Environment Impact of dairy manure pre-application treatment on manure composition , soil dynamics of antibiotic resistance genes , and abundance of antibiotic-resistance genes on vegetables at harvest', *Science of the Total Environment*. Elsevier B.V., 581–582, pp. 32–39. doi: 10.1016/j.scitotenv.2016.12.138.

Truchado, P., Gil, M. I., Moreno-Candel, M. and Allende, A. (2019) 'Impact of weather conditions , leaf age and irrigation water disinfection on the major epiphytic bacterial genera of baby spinach grown in an open fi eld', Journal Of Food Microbiology. Elsevier Ltd, 78(September 2018), pp. 46–52. doi: 10.1016/j.fm.2018.09.015.

Varshney, A., Das, M., Chaudhary, P., Kumari, R. and Yadav, K. (2018) 'Aeromonas Salmonicida as a Causative Agent for Postoperative', *Middle East African Journal of Ophthalmology*, 24(4), pp. 2–4. doi: 10.4103/meajo.MEAJO.

Warning, A. D. and Datta, A. K. (2017) 'Mechanistic understanding of non-spherical bacterial attachment and deposition on plant surface structures', Chemical Engineering Science. Elsevier, 160, pp. 396–418. doi: 10.1016/j.ces.2016.11.030.

Yeung, H., Chavarria, B. and Shahsavari, D. (2018) 'A Complicated Case of Serratia marcescens Infective Endocarditis in the Era of the Current Opioid Epidemic', *Case Reports in Infectious Diseases*, 2018, pp. 1–5.

Zekar, F. M., Granier, S. A., Marault, M.,Yaici, L., Gassilloud, B., Manceau, C., Touati, A. and Millemann, Y. (2017) 'From Farms to Markets : Gram-Negative Bacteria Resistant to Third-Generation Cephalosporins in Fruits and Vegetables in a Region of North Africa', *Frontiers in Microbiology*, 8(August), pp. 1–11. doi: 10.3389/fmicb.2017.01569.

Adenusi A, Abimbola W.A and Adewoga T.O.S. 2015. Human intestinal helminth contamination in pre-washed, fresh vegetables for sale in major markets in Ogun State, southwest Nigeria. Food Control 50: 843-849.

Akyar I, 2015. Food pathogens. Analytical chemistry 68: 617-648.

Amani J, Ahmadpour A, Fooladi AAI and Nazarian S. 2015. Detection of E. coli O157:H7 and Shigella dysenteriae toxins in clinical samples by PCR-ELISA. Brazil Journal of Infectious Diseases 19(3): 278-284.

Analysis of alkaline protease producing bacteria isolated from soil of India: Identification and detection of genetic variability. Journal of Genetic Engineering and Biotechnology 12: 27-35.

Aneja K, Dhiman R, Kumar N, Kumar V and Kaur M. 2014. Assessment of microbial proliferation and in vitro demonstration of the antimicrobial activity of the commonly available salad vegetables within Dhaka Metropolis, Bangladesh. International Journal of Food Science

Aworth C, 2015. Promoting food security and enhancing Nigeria's small farmers' income through value-added processing of lesser-known and under-utilised indigenous fruits and vegetables. Food Research International (2015), http://dx.doi.org/10.1016/j.foodres.2015.06.003.

Bartz JA, Yuk HG, Mahovic MJ, Warren BR, Sreedharan A and Schneider KR, 2015. Internalisation of Salmonella enterica in tomato fruit. Food Control 55: 141-150.

Burnett SL and Beuchat LR. 2001. Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. Journal of Industrial Microbiology and Biotechnology 27(2):104-110.

Campos J, Mourão J, Pestana N, Peixe L, Novais C and Antunes P. 2013. Microbiological quality of ready-to-eat salads: An underestimated vehicle of bacteria and clinically relevant antibiotic resistance genes. International Journal of Food Microbiology 166: 464–470.

Chen M, Wu Q, Zhang J, Yan Z and Wang J. 2014. Prevalence and characterisation of Listeria monocytogenes isolated from retail ready-to-eat foods in South China. Food Control 38:1-7.

Chitarra W., Decastelli L., Garibaldi A. and Gullino ML. 2014. Potential uptake of Escherichia coli O157:H7 and Listeria monocytogenes from growth substrate into leaves of salad plants and basil grown in soil irrigated with contaminated water. International Journal of Food Microbiology 189:139-145.

Dugassa A, Bacha K and Ketema T 2014. Microbiological quality and safety of some selected vegetables sold in Jimma town, South Western Ethiopia. African Journal of Environmental Science and Technology 8: 633-653.

Elhariry HM, 2011. Attachment strength and biofilm forming ability of Bacillus cereus on green-leafy vegetables: Cabbage and lettuce. Food Microbiology 28: 1266-1274.

Frasz SL and Miller JD, 2015. Fungi in Ontario maple syrup and some factors that determine the presence of mold damage. International Journal of Food Microbiology 207: 66-70.

Ge C, Lee C, Nangle ED, Li J, Gardner G, Kleinhenz M and Lee J. 2014. Impact of phytopathogen infection and extreme weather stress on internalization of Salmonella typhimurium in lettuce. International Journal of Food Microbiology 168-169: 24-31.

Gurler Z, Pamuk S, Yldirum Y and Ertas N. 2015. The microbial quality of ready-to-eat salads in Turkey: A focus on Salmonella spp and Listeria monocytogenes. International Journal of Food Microbiology 196: 79-83.

Hou MA, Grazia C and Malorgio G, 2015. Food safety standards and international supply chain organization: A case study of the Moroccan fruit and vegetable exports. Food Control 55:190-199.

Jaarsveld P, Faber M, Van Heerden I, Wenhold F, Van Rensburg W.J and Van Averbeke W. 2014. Nutrient content of eight African vegetables and their potential contribution to dietary reference intakes. Journal in food composition and analysis 33: 77-84.

Kongkachuichai R, Charoensiri R, Yakoh K, Kringkasemsee A and Insung P. 2015. Nutrients value and antioxidant content of indigenous vegetables form Southern Thailand. Food Chemistry 173: 838-846.

Kruger J, Mongwaketso T, Faber M, Van de Hoeven M and Smuts CM, 2015. Potential contribution of African green leafy vegetables and maize porridge composite meals to iron and zinc nutrition. Nutrition 31: 1117-1123.

Lund BM 2008. Bacterial Spoilage of Vegetables and Certain Fruits. Journal of Applied Bacteriology 34(1): 9–20.

Mosupye FM, Von Holy A. (2000). Microbiological hazard identification and exposure assessment of street food vending in Johannesburg, South Africa. International Journal of Food Microbiology, 61, 137–145.

Ntomola, S. S. (2014). Determination of Hygienic Practice and Status of Food Handlers in Catering Premises: A Case Study of Ilala Municipal Council(Doctoral dissertation, The Open University of Tanzania)

Nyenje M E, Collins E O, Nicoline F T, Ezekiel G and Roland N. 2012. Foodborne pathogens recovered from ready to eat Roadside Cafeterias, Retail outlets in Alice, Eastern Cape Province, South Africa. International Journal of Environmental Research and Public Health 9: 2608-2619

Oberholster P.J. and Botha AM. 2014. Importance of water quality to the food industry in South Africa. Understanding Food Energy Water Nexus. WWF-SA, South Africa. Available at: http://awsassets.wwf.org.za/downloads/5__a16269_water_quality_online. Accessed 2 May 2015).

Olaimat AN and Holley R, 2012. Factors influencing the microbial safety of fresh produce: A review. Food Microbiology 32: 1-19.

Patel J and Sharma M, 2010. Differences in attachment of Salmonella enterica serovars to cabbage and lettuce leaves. International Journal of Food Microbiology 139: 41–47.

Pilizota V, 2014. Fruits and Vegetables. Food Safety Management 9: 213-249.

Rekhy R, McConchie R. 2014. Promoting consumption of fruit and vegetables for better health. Have campaigns delivered on the goals? Appetite 79:113–123.

Ribera L.A, Palma MA, Paggi M, Knutson R, Masabni JG and Anciso J. 2012. Economic Analysis of Food Safety Compliance Costs and Foodborne Illness Outbreaks in the United States. Hort Technology 22(2): 150-156.

Rico D, Martin-Diana AB, Barat JM and Barry-Ryan C. 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: a review. Trends in Food Science & Technology 18:373–86.

Rodrigues FP, Begum M and Johannessen GS, 2014. Study of the cross contamination and survival of salmonella in fresh apples. International Journal of Microbiology 84: 92-97.

Sade E, Lassila, E and Björkroth J, 2015. Lactic acid bacteria in dried vegetables and spices. Food Microbiology 53: 110-114.

Sillani, S, and Nassivera, F, 2015 Consumer behavior in choice of minimally processed vegetables and implications for marketing strategies, Trends in Food Science & Technology, 2015 http://dx.doi.org/10.1016/j.tifs.2015.07.004.

Siroli S, Patrignani F, Serrazanetti DI, Tabanelli G, Montanari C, Gardini F and Lanciotti R. 2015. Lactic acid bacteria and natural antimicrobials to improve the safety and shelf-life of minimally processed sliced apples and lamb's lettuce. Food Microbiology 47: 74- 84.

Sospedra I, Rubert J, Soriano J M and Maries J. 2012. Microbial quality of plant-based foods served in Restaurants. Food Control Journal 30: 418-422.

Todd ECD, 2014. Personal hygiene and health. Food Safety Management 14: 769-798.

Tournas VH. 2005. Spoilage of Vegetable Crops by Bacteria and Fungi and Related Health Hazards. Critical review in microbiology 31(1): 33-44.

Van de Perre E, Deschuyffeleer N, Jacxsens L, Vekeman F, Van Der Hauwaert W, Asam S, Rychlik M, Devlieghere F and De Meulenaer B.2014. Screening of molds and mycotoxins in tomatoes, bell peppers, onions, soft red fruits and derived tomato products. Food Control 37: 165-170.

Van Wvk BE, 2011. The potential South African plants in the development of new food and beverage products. South African Journal of Botany 77: 887-868.

Vantarakis A, Affifi M, Kokkinos P, Tsibouxi M and Papapetropoulou. 2011. Occurrence of microorganisms of public health and spoilage significance in fruit juices sold in retail markets in Greece. Anaerobe 17(6): 288–291.

Wogu MD and Ofuase O. 2014. Microorganism responsible for spoilage of tomato fruits, Lycosperium esculentum, sold in Markets in Benin City, Southern Nigeria. Scholars Academic Journal of Biosciences 2(7): 459-466.

Xu G, Han Y, Li L, Liu J. Characterization and source analysis of indoor/outdoor culturable airborne bacteria in a municipal wastewater treatment plant. Journal of Environmental Sciences (China). 2018 Dec;74:71-78. DOI: 10.1016/j.jes.2018.02.007.

Zilelidou E A, Tsourou V, Loukou A and Skandamis P.N, 2014. Modelling transfer of E. coli 0157:H7 and L. monocytogenes during preparation of fresh cut salads: Impact of cutting and shredding practices. Food Microbiology 45:254-265.

Shobha S. 2014. Bacteriological analysis of Fresh vegetables and Fruits of local market and effect of Pretreatment by Antimicrobial agents on their Quality. International Research Journal of Biological Sciences 3(11):15-17.

## APPENDIX 1: ETHICS CLEARING LETTER

UNISA university of south africa

**CAES RESEARCH ETHICS REVIEW COMMITTEE**

Date: 07/04/2016

Ref #: **2016/CAES/053**
Name of applicant: **Ms S Ndlovu**
Student #: **57644101**

Dear Ms Ndlovu,

**Decision: Ethics Approval**

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**Proposal:** The microbiological quality of vegetables, fruits and their related processed products sold in formal and informal retail outlets

**Supervisor:** Dr F Tabit

**Qualification:** Postgraduate degree

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Thank you for the application for research ethics clearance by the CAES Research Ethics Review Committee for the above mentioned research. Final approval is granted for the duration of the project.

Please note point 4 below.

*The application was reviewed in compliance with the Unisa Policy on Research Ethics by the CAES Research Ethics Review Committee on 06 April 2016.*

*The proposed research may now commence with the proviso that:*

1) *The researcher/s will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.*
2) *Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study, as well as changes in the methodology, should be communicated in writing to the CAES Research Ethics Review Committee. An amended application could be requested if there are substantial changes from the existing proposal, especially if those changes affect any of the study-related risks for the research participants.*
3) *The researcher will ensure that the research project adheres to any applicable*

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*national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study.*

4) *Should the research discover that any of the vendors are selling contaminated food, the Ethics Committee must be informed. Subsequently, the College will inform the relevant local authority that dangerous levels of pathogens were found in a specific area. It is then up to the authority to start their own investigation to identify the vendors that sell the contaminated food. This approach will ensure that the researcher does not have to divulge his sources and thus compromise the confidentiality and anonymity of participants. The researcher must not deal directly with the relevant vendor in such a case.*

*Note:*
*The reference number [top right corner of this communiqué] should be clearly indicated on all forms of communication [e.g. Webmail, E-mail messages, letters] with the intended research participants, as well as with the CAES RERC.*

Kind regards,

Signature
CAES RERC Chair: Prof EL Kempen

Signature
CAES Executive Dean: Prof MJ Linington

NB point 4.



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## APPENDIX 2: PROPOSAL APPROVAL LETTER

UNISA university of south africa

Department of Life and Consumer Sciences
School of Agriculture and Life Sciences
College of agriculture and Environmental Sciences
Private Bag X6
Florida
1710

To: S Ndlovu (Student no: **57644101**)

**Subject: Outcome of your research proposal**

Your MSc research proposal titled:" **The microbial quality of vegetables, fruits and their related processed products**" has been approved by the departmental research committee.

You are advised to pay special attention to the comments raised by the review committee. These comments will be communicated to you by your supervisor.

Best regards

Lebelo ..... Date 14/03/2016

Dr SL Lebelo
COD:Department of Life and Consumer Sciences
Chair of the Department of Life and Consumer Sciences research committee